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CALIFORNIA AGRICULTURAL EXPERIMENT STATION

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THE DIGESTIBILITY OF BUR CLOVER AS AFFECTED BY EXPOSURE TO SUNLIGHT AND RAIN

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The principal forage plants of California foothill and valley ranges are annuals.[3] They germinate with the coming of the fall rains and make, during the winter, an amount of growth that varies according to moisture and temperature conditions. From February to May is usually the period of greatest growth. When the rains cease and moisture is depleted, the forage matures and dries. Stock is then either maintained on the dry feed or moved to summer ranges in the high mountains. In the latter case, the stock is brought to the lower ranges in the early fall and subsists on the old dry feed until rains bring on new forage.

The changes in the plants from the early vegetative stage to the dried condition involve marked changes in chemical composition and nutritive value. After drying, the feed is subjected to the processes of weathering.

Studies by Woodman and others(1, 2, 3) on the nutritive value of pasture have shown that young pasture grass is in digestible composition a "watered concentrate" rather than a roughage. They found that 70 per cent of the organic matter was digestible and that the small amount of fiber which it contained was 80 per cent digestible. The immature grass contained approximately 20 per cent digestible protein with a nutritive ratio of about 1:3. As plants approach

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[3] The most common of the grasses are several species of brome, wild oats (*Avena fatua*), fescue grass (*Festuca megalura*), and foxtail (*Hordeum murinum*). Bur clover (*Medicago hispida*) and alfilaria (*Erodium* sp.) are found on the better ranges. Salt grass (*Distichlis spicata*), a *perennial*, is also important in some areas.

maturity, the percentage of nitrogen-free extract and fiber increases, while the protein decreases. The result is a widening of the nutritive ratio and a decrease in digestibility.

Cattle do not graze extensively on green bur clover when other forage such as grasses and alfilaria are present in adequate amounts. As soon as the forage matures and dries, however, they show a decided preference for bur clover.

A high forage value is generally recognized in bur clover ranges during the first few weeks after the feed has cured, and the final finish is usually made by fattening cattle during this period. A late rain, coming after the feed is cured, is disastrous from the standpoint of finishing cattle without supplement; and under such conditions it has, in many cases, been found difficult even to maintain breeding stock.

The most efficient utilization of range forage is a problem involving the proper supplementing of this feed in such a way as to keep the animals supplied at all times with a well balanced diet. To accomplish this it is necessary to have definite information on the changes in composition of the feed and its effect on the nutrition of the animal. Since bur clover is an important forage species which maintains good feeding qualities in the cured condition, the effect of weathering upon its nutritive value is of particular interest.

DIGESTION EXPERIMENTS WITH BUR CLOVER

Approximately one acre of a nearly pure stand of bur clover was cut May 17, 1927, the only contamination being a few star thistles. Most of the burs were still green at the time of cutting, but the seeds were well formed and most of them were yellow in color after the forage had been dried.

Immediately after cutting, the clover was spread out in a thin layer on a clean, concrete pavement and exposed to direct sunlight. It dried rapidly, and on the following day a portion was piled in small cocks for further curing. On the fourth day a rain storm, lasting a few minutes, necessitated the placing of this material in larger piles to minimize wetting. Only the tops and bottoms of these piles were wet, and apparently the shower did little damage. The following day the clover was turned and allowed to cure in large cocks until the seventh day after cutting, at which time it was chopped, thoroughly mixed, sacked, and stored in a dark loft. This portion was bright green in color and was designated as bur clover No. 1.

The remainder of the clover was allowed to dry and bleach in the sun. It was spread out in a thin layer and was mixed and turned at intervals of a few days in order to expose all the material to the sun and simulate field conditions as nearly as possible. During this period (May 17 to June 7) two showers totaling 0.31 of an inch of rain fell. On June 7 another rain storm threatened. One-half of the clover was therefore put under cover and later chopped, mixed, sacked, and stored. This was designated as bur clover No. 2. In contrast to bur clover No. 1, it was brown in color.

This second rain, which amounted to 0.47 inch, fell on the clover remaining after lot 2 had been removed. The water which drained from the clover was decidedly brown in color. The leaching effect of this rain was more noticeable than that of the two previous lighter showers. After the rain, this material was left exposed for an additional 14 days; then it was chopped, mixed, sacked, and stored. It was designated as bur clover No. 3. The treatment of each of the lots may be briefly summarized as follows:

Bur clover No. 1. Cured for one day in a thin layer, then cured in cocks for six days.

Bur clover No. 2. Exposed in a thin layer for 21 days, during which time it was wet twice by rain totaling 0.31 inch.

Bur clover No. 3. Exposed in a thin layer for 34 days, during which time it was wet three times by rain totaling 0.78 inch.

The original plan was to study the influence of varying periods of exposure to sunlight, upon digestibility. The late rains provided an opportunity to study the influence of this additional factor upon the feed, but it was unfortunate that the study of lot 2 was complicated by wetting, as it eliminated the possibility of comparing directly the relative influence of exposure to sunlight and to rain.

During March, April, and May, 1928, digestion experiments were conducted on these three lots of bur clover. The same five wether sheep were used in each of the trials. They were fed an amount of bur clover which was calculated to be sufficient for maintenance, the value assumed being similar to that of average alfalfa hay. The methods used in conducting these experiments were fully discussed in an earlier publication(4) and are summarized here.

The animals were placed in individual box stalls 4 feet by 8 feet, equipped with mangers so constructed as to prevent any possible loss of feed. The feces were collected by means of rubber-lined sacks attached to each animal.

The preliminary feeding period was 10 days and the collection period 15 days.

An amount of bur clover sufficient to last throughout a digestion trial was thoroughly mixed and spread out on a clean concrete floor. The individual feeds for the entire period were then weighed out into paper bags. The bags were labeled designating the animal to which the feed contained was to be given. To obtain a sample for chemical analysis a large quantity was taken and reduced to about ¼ bushel by mixing and quartering. This amount was then ground in a hammer mill, thoroughly mixed, and the final sample for chemical analysis taken from the fine material. A sample for moisture determination was taken before grinding.

The collection bags were emptied twice daily. The feces were immediately weighed and aliquot portions of the feces of each animal were placed in glass mason jars which had been previously rinsed in a 10 per cent alcoholic thymol solution. In addition, powdered thymol was sprinkled over the feces after they were transferred from the scales to the jar to the amount of 5 grams to each jar. The jars were immediately placed in a refrigerator where they were maintained at a temperature varying from 28 to 35 degrees Fahrenheit. At the end of the collection period the contents of the several jars representing the total feces collected from each animal were thoroughly mixed, ground, remixed and sampled for chemical analysis.

The data from the digestion trial with bur clover No. 1 are given in tables 1, 2, and 3.

TABLE 1

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 1, grams	Feces grams
137	9,600	7,457.5
139	12,000	9,722.5
717	10,500	8,589.5
138	7,500	5,349.5
135	11,700	10,556.0

TABLE 2

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 1

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	38.85	5.00	13.88	1.63	12.72
Sheep No. 139	38.01	4.68	14.73	1.60	11.56
Sheep No. 717	38.46	5.22	14.43	1.70	11.69
Sheep No. 138	43.34	5.63	17.15	1.66	13.11
Sheep No. 135	35.12	4.32	13.71	1.21	11.19
Bur Clover No. 1	86.62	15.34	40.65	2.89	19.90

TABLE 3

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 1

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	65.16	74.68	73.48	56.19	50.35
139	64.45	75.18	70.64	55.14	52.93
717	63.68	72.27	70.96	51.88	51.94
138	64.31	73.82	69.91	59.03	53.07
135	63.42	74.59	69.57	62.22	49.27
Average	*64.20*	*74.11*	*70.91*	*56.89*	*51.51*

The data from the digestion trial with bur clover No. 2 are given in tables 4, 5, and 6.

TABLE 4

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 2 grams	Feces, grams
137	8,353.0	7,072.0
139	11,866.0	10,355.0
717	9,667.0	8,398.5
138	7,387.0	5,996.5
135	11,491.0	10,300.0

TABLE 5

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 2

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	41.16	5.39	16.93	1.47	12.69
Sheep No. 139	40.28	5.36	16.57	1.48	12.22
Sheep No. 717	41.97	6.28	16.88	1.58	12.22
Sheep No. 138	44.66	6.03	17.89	1.66	13.82
Sheep No. 135	39.98	5.23	16.28	1.46	12.22
Bur Clover No. 2	87.48	15.18	40.48	2.22	21.97

TABLE 6

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 2

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	60.17	69.88	64.59	43.91	51.10
139	59.82	69.19	64.28	41.80	51.46
717	58.32	64.06	63.77	38.16	51.68
138	58.56	67.75	64.12	39.33	48.94
135	59.04	69.12	63.95	41.04	50.14
Average	*59.18*	*68.00*	*64.14*	*40.85*	*50.66*

The data from the digestion trial with bur clover No. 3 are shown in tables 7, 8, and 9.

TABLE 7

TOTAL FEED CONSUMED AND TOTAL FECES COLLECTED

Sheep No.	Bur clover No. 3 grams	Feces, grams
137	8,700.0	8,181.0
139	12,000.0	12,658.5
717	9,750.0	8,808.5
138	7,500.0	6,589.5
135	11,700.0	11,312.0

TABLE 8

CHEMICAL ANALYSES OF FECES AND OF BUR CLOVER NO. 3

Feces	Dry matter per cent	Crude protein per cent	Nitrogen-free extract per cent	Ether extract per cent	Crude fiber per cent
Sheep No. 137	41.78	5.98	16.90	1.60	12.62
Sheep No. 139	38.53	5.57	15.72	1.45	11.49
Sheep No. 717	45.02	7.08	18.39	1.82	12.41
Sheep No. 138	46.43	6.69	18.69	1.77	13.99
Sheep No. 135	41.99	5.96	17.06	1.53	12.51
Bur Clover No. 3	91.15	16.28	40.87	2.02	25.04

TABLE 9

COEFFICIENTS OF DIGESTIBILITY OF BUR CLOVER NO. 3

Sheep No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
137	56.90	65.46	61.12	25.51	52.61
139	55.41	63.91	59.43	24.28	51.60
717	55.38	60.71	59.35	18.60	55.23
138	55.25	63.90	59.82	23.02	50.91
135	55.46	64.60	59.64	26.77	51.70
Average	*55.68*	*63.72*	*59.87*	*23.64*	*52.41*

Tables 3, 6, and 9 show the percentage of each ingredient in the three lots of bur clover digested by the animals. As separate data were obtained from each animal, the average represents the results of five separate trials. The variation of individual sheep from the average of each trial was very small. The greatest variation is found in the percentage of ether extract digested, which is the nutrient present in smallest amounts and is therefore subject to the greatest amount of experimental error. The variation of the ether extract from the average is not very great and has little influence upon the total digestible nutrients in the feed.

1.19
19.90

There was some variation in the moisture content of the three lots of bur clover, and therefore a comparison can best be made upon the dry basis. The chemical composition of the three lots on the dry basis is given in table 10.

TABLE 10

PERCENTAGE COMPOSITION OF BUR CLOVERS 1, 2, AND 3; DRY BASIS

Bur Clover No.	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Total ash	CaO	P_2O_5
1	17.71	46.93	3.34	22.97	9.05	......	0.72
2	17.35	46.27	2.54	25.11	8.72	1.24	0.68
3	17.86	44.84	2.22	27.48	7.61	1.30	0.67

The difference in composition between bur clover No. 1 and No. 2 does not appear very significant except for the lower ether extract and the slightly higher crude fiber in No. 2. Perhaps the processes involving the change in color of the chlorophyll and loss of aromatic compounds may have affected the amount of ether-extractable material.

Bur clover No. 3 is slightly higher in protein than No. 1 and lower in nitrogen-free extract, ether extract, and total ash. The decrease in nitrogen-free extract and ash, with the corresponding increase in crude fiber, may be taken as indicative of leaching.

The average coefficients of digestibility of each nutrient in the three lots of bur clover are shown in table 11.

TABLE 11

AVERAGE COEFFICIENTS OF DIGESTIBILITY

Bur Clover No.	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber
1	64.20	74.11	70.91	56.89	51.51
2	59.18	68.01	64.14	40.85	50.66
3	55.68	63.72	59.87	23.64	52.41

Table 11 shows that from bur clover No. 1 to No. 3 there was a progressive decrease in digestibility of all nutrients except crude fiber.

The extent to which the decrease in digestibility could be attributed relatively to leaching by rain or to changes resulting from other factors was not known, since the difference in chemical composition was not very great. It is possible, however, that considerable amounts of each nutrient, with the exception of crude fiber, might have been extracted and still not have changed very greatly the composition of the residue. Since part of the soluble material had already been removed from bur clovers 2 and 3, it was expected that if samples

of all three lots were subjected to leaching under identical conditions in the laboratory that the difference in amount of material extracted would indicate the extent of the loss by rain, providing other factors such as exposure to sunlight and air had not changed the solubility of the nutrients. Accordingly, approximately 400-gram samples each of bur clovers 1, 2, and 3 were taken for the leaching experiments. The burs were separated from the stems and leaves, and the percentage of each was determined. The percentage of burs was 31.4 per cent, 30.8 per cent, and 31.2 per cent for samples 1, 2, and 3, respectively. The stems and leaves were thoroughly mixed and divided into two approximately equal portions. Each sample was then made up to exactly 30 per cent burs and 70 per cent leaves and stems. One portion was used for analysis, the other was leached. The weights of samples leached were 172 grams, 168 grams, and 182 grams respectively, for bur clovers 1, 2, and 3. The samples were placed in soil percolators for one hour with two liters of distilled water; they were then washed twice with one-liter portions of water, and the final volume of extract was made up to four liters. The extract was first filtered with suction through linen, and the portions used for analysis were filtered through filter paper to remove any solids in suspension. The percentage of the total dry matter extracted was determined and found to be 19.94, 15.97, and 11.73 for bur clovers 1, 2, and 3, respectively.

In order to ascertain whether exposure to sunlight and air without leaching would bring about chemical changes which would decrease the amount of soluble material, a quantity of bur clover was collected and dried by spreading out in a thin layer on canvas for 2½ days. One-half was then stored and the other allowed to bleach in the sun for 40 days. It was protected against loss of leaves by screens and was taken indoors when the weather was inclement. At the end of this time it was very dry and thoroughly bleached. Duplicate 100-gram samples of each lot were then leached under identical conditions. No difference was found in the amount of total solids extracted.

In another experiment in which samples of alfalfa meal were extracted with water after exposure to irradiation from a quartz mercury vapor lamp for 2 hours at a distance of 18 inches, no difference in water soluble material was found. It was therefore concluded that exposure to light and air did not effect the solubility of the nutrients in forage and that the difference found between the different lots of bur clover was caused by the previous leaching by rain.

The difference in digestible organic matter per 100 pounds of dry matter between bur clover No. 1 and No. 2 was 4.89 pounds. The difference between No. 1 and No. 3 was 6.95 pounds. The amount of organic matter indicated to have been lost from bur clover No. 2 and No. 3 through the action of rain was 3.2 and 6.5 pounds, respectively. If this soluble organic matter is assumed to be highly digestible the greater part of the difference in digestibility can be accounted for by the loss of these soluble constituents.

The digestible nutrients in 100 pounds of dry matter in bur clovers 1, 2, and 3 are shown in table 12.

TABLE 12

POUNDS OF DIGESTIBLE NUTRIENTS IN 100 POUNDS OF DRY MATTER

Bur clover No.	Crude protein	Carbohydrate	Fat	Total*	Nutritive ratio
1	13.13	45.11	1.89	62.49	1:3.68
2	11.80	42.40	1.04	56.54	1:3.79
3	11.41	41.25	0.52	53.83	1:3.72

* Total includes fat times the factor 2.25.

The total digestible nutrients decreased from 62.5 in bur clover No. 1 to 56.5 and 53.8 in bur clover No. 2 and No. 3, respectively. This represents a decrease in total food value of 9.54 per cent in No. 2 and of 13.8 per cent in No. 3, compared to bur clover No. 1. The ratio of protein to carbohydrate and fat remained practically unchanged and is relatively narrow.

Bur clover No. 2 and No. 3 were apparently less palatable to the sheep than was bur clover No. 1. Upon changing from the latter to No. 2 it was found necessary to reduce slightly the quantity fed in order to induce the sheep to consume the entire ration.

In spite of a significant decline in total digestible nutrients, bur clover No. 3 was still comparable in digestible composition to average alfalfa hay.

Since the bur clover was cured on concrete floors, where it was possible to recover all of the burs, stems, and leaves, each lot was representative of the entire plant as it occurred in the field. The chemical composition of the burs as compared with the stems and leaves is shown in table 13.

With the exception of the ash there is no very significant difference in the composition of burs and of stems and leaves. It would, therefore, seem doubtful that the total feed value of the burs is any greater than that of the stems and leaves combined, especially as large numbers of seeds were observed to be practically unchanged in the feces.

TABLE 13

PERCENTAGE COMPOSITION OF BURS AND OF STEMS AND LEAVES; DRY BASIS

	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Total ash
Burs	15.12	51.42	3.33	23.78	6.35
Stems and leaves	16.33	47.11	2.36	23.81	10.39

The net energy value in therms per 100 pounds dry matter for each of the three lots of bur clover has been computed according to the method of Armsby[5] and is given below:

Bur clover No. 1—43.18 therms.

Bur clover No. 2—35.35 therms.

Bur clover No. 3—32.06 therms.

According to Armsby the maintenance requirement for a 1,000-pound steer is 6 therms of net energy daily, and the average requirement for each pound of increase during fattening is 3.25 therms. The significance of the difference in digestible composition of the three lots of bur clover may be demonstrated by a hypothetical case wherein a 1,000-pound steer eats 25 pounds of bur clover daily. The gain expected from each of the lots of bur clover has been computed and is shown in table 14.

TABLE 14

COMPUTED NET ENERGY VALUE OF THE FEED AND GAIN IN LIVE WEIGHT FROM THE CONSUMPTION OF 25 POUNDS OF DRY MATTER DAILY

Bur clover No.	Total net energy therms	Required for maintenance therms	Available for gain therms	Computed gain pounds
1	10.80	6	4.80	1.47
2	8.84	6	2.84	0.87
3	8.01	6	2.02	0.62

If the total dry matter consumed daily in each case were limited to 20 pounds, the computed gains would be approximately 0.8 pound, 0.3 pound, and no gain, respectively, for bur clovers 1, 2, and 3.

Table 14 shows that even a comparatively small change in total feed value reduces the margin of energy above the maintenance requirement so that gains are seriously affected.

The effect of excessive exposure and of rain is probably minimized in this experiment because all the burs and leaves were saved. On the range there undoubtedly would be a heavy loss of leaves because of the beating effect of the rain and because of the tendency of the leaves subsequently to become brittle, easily pulverized, and hence lost by being mixed with dirt or blown away by the wind. The loss of leaves would probably cause a marked decline in protein, ash, and

digestible carbohydrate. Table 15 from Henry and Morrison[6] shows the relative composition of alfalfa hay, leaves, and stems. The difference in composition probably holds true in a general way for bur clover leaves and stems.

TABLE 15

THE PERCENTAGE COMPOSITION OF ALFALFA HAY, LEAVES AND STEMS

	Water	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Alfalfa hay	8.6	14.9	37.3	2.3	28.3	8.6
Alfalfa leaves	6.6	22.5	41.2	3.4	12.7	13.6
Alfalfa stems	5.6	6.3	27.9	0.9	54.4	4.9

Table 15 shows that the alfalfa leaves contained 22.5 per cent protein as compared to 6.3 per cent in the stem. The leaves were also much higher in easily digestible carbohydrate and very much higher in ash. This indicates that any condition which results in loss of leaves would cause a decided decrease in forage value.

SUMMARY

Bur clover, in common with other legumes, is rich in protein and has a narrow nutritive ratio. Even when cut in advanced stages of maturity it has a higher coefficient of digestibility than most hays.

Weathering of bur clover, which included exposure to rain, resulted in a decrease in digestibility of each nutrient except crude fiber. Evidence has been presented which indicates that the loss of soluble constituents caused by rain may have been responsible for the greater part of the decrease in digestibility.

The bleaching and leaching processes apparently decreased the palatability of the bur clover used in the digestion experiments.

The significance of the decrease in digestibility on gains in live weight has been discussed in the text.

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THE EFFECT OF LEACHING ON THE NUTRITIVE VALUE OF FORAGE PLANTS[1]

H. R. GUILBERT[2] S. W. MEAD,[3] AND H. C. JACKSON[4]

INTRODUCTION

A significant decrease in digestibility of bur clover after exposure to sunlight and rain has been reported in the first paper and evidence was presented which indicated that the greater part of the decrease could be accounted for by the loss of soluble constituents through the action of rain. Field observations support these findings.

After late rains on cured range feed, cattle have been observed to cease gaining and to require supplemental feeding in order to fatten sufficiently to be marketable. Under such conditions it is difficult, in many cases, even to maintain breeding stock. This situation prevailed over a large area of California in 1929. Extensive supplemental feeding was required in many areas to fatten the cattle for beef, and stock cattle, generally, suffered from the poor feed. Among the abnormal conditions reported in cattle from some areas were pica, particularly bone craving, deformed calves, difficult parturition, and retained placenta. Many ewes which apparently were unable to lactate abandoned their lambs. The indications are that these troubles were directly associated with the poor quality of the feed.

Rain followed by warm, humid weather is favorable to the development of molds. Frequently, however, the feed dries quickly with little or no molding; and yet deterioration has occurred, as evidenced by the condition of livestock. Field observations therefore indicate that the leaching effect of rain may be the most important factor in the loss of nutritive value.

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REVIEW OF LITERATURE

Wolff[1] in Germany reported that 20 per cent of the dry substance of hay may be lost by simply soaking in cold water, and that clover hay suffers from rain more than meadow hay because from 25 to 40 per cent of its dry substance may be dissolved. He reported analyses by Ritthausen at Möckern on two samples of clover hay which were cut at the beginning of the flowering period from the same field at the same time. One was quickly dried and the other left to lie for two weeks exposed to intermittent rains. The percentage composition was reported as follows:

	Water	Crude protein	Nitrogen-free extract and fat	Crude fiber	Ash
Not rained upon	16.0	14.6	36.1	25.3	8.0
Rained upon	16.0	15.8	23.4	37.4	7.5

The principal change was a decrease in the most soluble carbohydrates and ash and a resultant increase in crude fiber.

Headden[2] of the Colorado Experiment Station reports the percentage composition of alfalfa hay before and after being exposed to rain as follows:

	Protein	Nitrogen-free extract	Crude fat	Crude fiber	Ash
Not rained upon	18.71	38.71	3.94	26.46	12.18
Rained upon	11.01	33.64	3.81	38.83	12.71

The latter hay was damaged by three rains at intervals of two days or more. The author states: "The mechanical loss of leaves and stems would tend to change the composition of the hay in the direction indicated by the analyses, but for good reasons we do not consider this to enter largely into this particular case, but attribute the changes in composition to the action of heat and moisture."

Henry and Morrison[3] state: "Exposure to the sun reduces the palatability by bleaching and causes a loss of aromatic compounds, dew works injury and rain carries away the more soluble portions. The actual damage from rain is even greater [than analysis shows], for the nutrients lost were those most soluble and hence most easily digested." According to Piper[4] the destruction of the green chlorophyll by sunlight is increased by the action of dew. He also makes the following statement:

"Westgate sprinkled perfectly cured crimson clover hay with water to imitate rain for one hour on each of three successive days. On analysis it was found in comparison with a sample unsprinkled to have lost about three-fourths of its sugar, one-ninth of its protein and three-fourths of its ash constituents."

Le Clerc and Breazeale[5] harvested a sample of greenhouse barley at the heading period and subjected the whole plant to leaching. They state: "The plant was placed in a large evaporating dish and soaked with water for several minutes. After drying, this operation was again repeated. The plant was then dried and analyzed. The washings were also analyzed, the results showing that 1.6 per cent of the whole nitrogen of the plant was lost on washing or soaking, 36 per cent of the phosphoric acid, 65 per cent of the potash, 52 per cent of the soda, 45 per cent of the magnesia, and 75 per cent of the chlorin."

Various other plants, among them rice, wheat, apple twigs, oats, and potatoes, were experimented upon in a number of ways and at different stages of growth. The largest loss of nutrients by leaching was found to occur when the plant was at maturity. In the growing state, however, some losses occur. When wheat plants were in bloom, the amounts washed out of the straw and leaves were as follows: "Nitrogen, 1.4 per cent; phosphoric acid, nothing; potash, 4.4 per cent; soda, 12.7 per cent; lime, nothing; magnesia, 10.3 per cent; chlorin, 7.6 per cent." From this Le Clerc and Breazeale state: "It is not contended that the green plants give off very much of their plant food by such treatment, for it is very probable that most of the ash ingredients removed by washing are those which were in the dead or wilted tissue, as it is well known that when plants dry or wilt, the inorganic constituents exude to the surface, where they may be easily washed off if subjected to the action of rain, dew, etc. As illustrative of this, an experiment made with freshly cut grass showed that when the grass was dried previous to treatment with water a much larger amount of ash materials was washed out. This explains why it is that when freshly cut hay has been rained upon it is only slightly injured, whereas if the rain comes after the hay has been dried the loss is considerable, sometimes as much as half of the ash ingredients being thus removed."

Digestion experiments[6] and field observations indicate that the leaching effect of rain is an important factor in loss of nutritive value. Although the effect of leaching is indicated in the literature cited, these experiments do not appear directly applicable to range conditions. Experiments were therefore undertaken with species of forage

plants common to California ranges for the purpose of showing the extent and character of the losses which could be caused by rain, and thus contribute further information on the reasons for the observed deterioration in nutritive value.

LEACHING EXPERIMENTS WITH BUR CLOVER

A quantity of bur clover was cut at an advanced stage of maturity but was still green in color. It was dried in the sun for one day in a thin layer and then cured for 6 days in cocks. This was the lot designated as No. 1, in the digestion experiments reported in the first paper.

For the leaching experiment a sample weighing 172 grams was placed in a soil percolator for one hour with two liters of distilled water; it was then washed twice with one-liter portions of water, and the final volume of the extract made up to four liters. The extract was first filtered with suction through linen, and the portion used for analysis was filtered through filter paper to remove any solids in suspension. The results are summaried in table 1.

TABLE 1

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM BUR CLOVER NO. 1; DRY BASIS

Crude protein	16.20
Nitrogen-free extract	28.32
Calcium	30.43
Phosphorus	45.71
Chlorine	86.02
Total silica-free ash	59.11
Total solids	19.94

As shown in table 1, nearly 20 per cent of the dry matter of the forage was extracted by water. The silica-free ash, the various ingredients of the ash, and the nitrogen-free extract were most susceptible to leaching. Although the percentage loss of protein was least, the amount extracted appears significant particularly since the digestion experiments show a decreased availability of the protein after exposure to weathering and to rain.

Although the nutritive value of bur clover is markedly affected by rain, it is still much higher than that of the dried grasses and other forage under similar conditions; and stock can be maintained fairly

well so long as the clover burs are obtainable in adequate quantities. The relative effect cf leaching on the burs and the enclosed seeds as compared with the remainder of the plant is, therefore, of interest, and another experiment was carried out to determine this.

A sample from another lot of bur clover was used in this experiment. The burs were separated from the stems and leaves, and samples of each were thoroughly mixed and quartered, opposite quarters being taken for leaching and analysis. The samples for leaching were weighed and placed in soil percolators, which were then filled with distilled water and allowed to stand for approximately one hour. The extract was then drawn off, and the residue washed with distilled water until it came off practically colorless. The extract was then made up to the nearest convenient volume. A portion of each of the original samples and of the extracts were analyzed. The results are shown in table 2.

TABLE 2

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM CLOVER BURS AS COMPARED TO STEMS AND LEAVES; DRY BASIS

	Clover burs	Leaves and stems
Crude protein	9.3	11.2
Nitrogen-free extract	15.3	35.3
Calcium	9.5	19.6
Phosphorus	16.3	58.7
Total silica-free ash	26.5	34.4
Total solids	10.8	21.7

The clover burs were much more resistant to leaching than the stems and leaves, the loss of total solids being only half as great. The difference in the percentage of calcium, phosphorus, and carbohydrate extracted is especially large.

LEACHING EXPERIMENT WITH OAT HAY

A sample of good quality red-oat hay was cut into 2 to 3-inch lengths, and a weighed quantity placed in a soil percolator with distilled water and allowed to stand for approximately one hour. The extract was then drawn off and the residue washed with distilled water until it came off practically colorless. The extract was filtered and made up to a convenient volume. Samples of the original, of the residue after leaching, and of the extract were analyzed.

The percentage of chemical constituents extracted is shown in table 3.

TABLE 3

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM RED-OAT HAY; DRY BASIS

Crude protein	1.1
Nitrogen-free extract	14.2
Calcium	31.3
Phosphorus	21.4
Chlorine	67.2
Total silica-free ash	59.2
Total solids	10.4

The loss of total solids was less than from bur clover under similar conditions of extraction. The oat hay was lower in protein, and a small amount was removed by leaching. The percentage of carbohydrate soluble in water was also less than in the clover. The loss of ash, however, was nearly 60 per cent of the total.

LEACHING EXPERIMENTS WITH NATURALLY-CURED RANGE FORAGE

All of the previous experiments were conducted on samples cut in the green stage and dried. Because changes in composition occur during the latter stages of maturity, it appeared desirable to experiment with samples of naturally-cured range feed. The following is a description of the samples used:

Sample No. 200 was soft chess (*Bromus hordeaceus*). It was dry and bleached, and the seeds were mostly shattered. The sample was collected on June 3, 1930.

Sample No. 212 was a composite in which stork's-bill alfilaria (*Erodium botrys*) predominated. It was collected on June 5, 1930; it was dry and bleached, and the seeds were completely shattered.

Sample No. 215 was dry, bleached bur clover (*Medicago hispida*). Many of the burs had fallen to the ground, but a large percentage of these were included in the sample in order to have it as nearly representative of the material grazed as possible. It was collected on June 9, 1930.

All the samples were ground to pass through a 40-mesh screen. Fifty-gram samples of each were placed in flasks with 500 cc. of distilled water and allowed to stand for approximately one hour. The

extracts were filtered with suction and the residues washed with 150 to 200 cc. of distilled water. The final volume of extract varied from 500 to 590 cc. The purpose of the experiment was to ascertain whether or not the relative loss of the various nutrients would be similar to that found in previous experiments.

The percentage of each nutrient extracted is shown in table 4.

TABLE 4

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM NATURALLY-CURED FORAGE; DRY BASIS

	No. 200, soft chess	No. 212, alfilaria	No. 215, bur clover
Crude protein	18.2	12.0	12.9
Nitrogen-free extract	12.1	12.6	15.0
Calcium	30.5	11.8	9.5
Phosphorus	37.0	45.4	31.9
Chlorine	72.6	63.0	76.9
Total silica-free ash	62.7	28.3	32.8
Total solids	10.7	8.0	10.6

The greatest percentage loss was in the ingredients of the silica-free ash, a fact which is in agreement with the other experiments. The percentage loss of ash from soft chess was approximately double that from alfilaria and bur clover. Compared with all the previous experiments, a higher percentage of protein relative to other ingredients was extracted.

Another lot of four naturally-cured samples, each weighing 25 grams, was prepared in the same way as the previous samples and extracted with distilled water for 5 hours in a Soxhlet apparatus. At the end of this time the water which came over was colorless. The treatment of these samples probably approached complete extraction. A brief description of the samples follows:

No. 221. Soft chess, dry, bleached, and the seeds partly shattered.

No. 222. Wild oats, dry, bleached, and the seeds mostly shattered.

No. 226. Bur clover, dry, bleached, and consisting largely of burs and some stems. Most of the leaf material had been shattered and lost.

No. 228. Stork's bill alfilaria, dry, bleached, and the seeds shattered.

All of the samples were collected in the same locality on June 10, 1930. They were taken from a different area than the samples reported in table 4, and had been dry somewhat longer. The results of the Soxhlet extraction are shown in table 5.

TABLE 5

PERCENTAGE OF CHEMICAL CONSTITUENTS EXTRACTED FROM NATURALLY-CURED FORAGE WITH SOXHLET APPARATUS; DRY BASIS

	No. 221, soft chess	No. 222, wild oats	No. 226, bur clover	No. 228, alfilaria
Crude protein	6.8	11.8	14.3	12.2
Nitrogen-free extract	6.1	8.9	15.6	17.1
Calcium	26.3	47.2	10.2	11.7
Phosphorus	18.8	24.6	27.7	42.5
Total silica-free ash	30.0	66.9	34.8	24.6
Total solids	5.0	8.3	11.9	12.1

The loss of total solids varied from 5 to 12 per cent, and the greatest percentage loss was in the silica-free ash. There was a relatively greater loss of calcium than of phosphorus in wild oats and soft chess, whereas the reverse was found in bur clover and alfilaria. The variation in chemical composition between this lot of samples and the first lot of naturally cured forage, may account for some of the variations in leaching. The soft chess sample, No. 221, for example, was significantly lower in protein and nitrogen-free extract and higher in fiber than sample No. 200. The general trend of the results is the same as in previous experiments.

The results of this experiment indicate that the lower amount of total solids extracted in the range samples as compared to bur clover No. 1 results not so much from the method of leaching as from a lower content of soluble material. The loss of total solids in the wild oats and in soft chess, sample No. 200, was not far from that found in red-oat hay, table 3. The loss from bur clover sample No. 226 was similar to that found for a pure sample of burs, table 2. Because of the shattering of leaves under field conditions, the bur clover and alfilaria samples used in these experiments are not representative of the entire plant. It is not possible, therefore, from the data available, to compare directly the solubility of nutrients in forage cut in advanced stages of maturity but still green, with the fully matured and naturally dried forage.

THE COMPOSITION OF RESIDUES AFTER LEACHING

The foregoing data have shown the percentage loss of the various nutrients through leaching and hence indicate the possible loss in tonnage of cured feed. The utilization of the material which is left, however, is a most important consideration. The composition of the residues are shown in tables 6, 7, and 8.

TABLE 6

COMPARISON OF THE PERCENTAGE COMPOSITION OF UNLEACHED PORTIONS OF BUR CLOVER AND RED-OAT HAY WITH THE RESIDUES AFTER LEACHING; DRY BASIS

	Bur clover No. 1*		Bur clover burs		Bur clover stems and leaves		Red-oat hay	
	Unleached sample	Leached residue	Unleached sample	Leached residue†	Unleached sample	Leached residue†	Unleached sample	Leached residue
Crude protein	16.86	17.77	15.12	15.38	16.33	18.51	5.15	5.62
Nitrogen-free extract	45.54	41.67	52.21	49.73	46.93	38.76	60.35	57.68
Ether extract	3.31	3.94	2.54	2.85	2.54	3.24	3.43	3.83
Crude fiber	25.31	31.25	23.78	26.66	23.81	30.41	25.00	28.22
Silica-free ash	7.56	3.89	5.84	4.81	9.53	7.98	3.07	1.08
Acid-insoluble ash	1.42	1.48	0.51	0.57	0.86	1.10	3.00	3.57
Calcium	0.95	0.94	0.83	0.84	1.20	1.23	0.23	0.18
Phosphorus	0.30	0.22	0.40	0.38	0.20	0.10	0.18	0.16
Chlorine	0.60	0.20	......	......	......	......	0.76	0.29

* Results in the first two columns were obtained from a different sample from that reported in table 1.

† Computed from the original and the extract.

Nitrogen-free extract represents a large percentage of the total dry matter in the plant. In the leaching experiments shown in table 6 there was a considerable loss of this constituent, which has the effect of increasing the percentage of other ingredients of the residue in which the percentage loss is less.

The protein in the residue after leaching was in every case higher than in the original material. The nitrogen-free extract was lower in the residue in every case, while the ether extract and crude fiber were higher than in the unleached sample. The silica-free ash was reduced in every instance, and in the oat hay it was reduced to a little more than 1 per cent, which is extremely low.

Of the ingredients of the ash, calcium was least affected by leaching except in red-oat hay, where the calcium loss was greater than that of phosphorus. The phosphorus of bur clover No. 1 was reduced from 0.30 per cent to 0.22 per cent. In the case of the bur clover leaves and stems, the amount was reduced to 0.10 per cent, which is definitely low. Animals grazing on leached bur clover in which the per cent of burs eaten is less than in these samples may be ingesting less than optimum amounts of phosphorus.

The ratio of calcium to phosphorus in bur clover No. 1 was changed from approximately 3:1 to 4.5:1, while in the stems and leaves it was changed from 6:1 to 12:1. The clover burs contained twice as much phosphorus as the stems and leaves, and the ratio of calcium to phosphorus was not affected by leaching. The quantity of burs available may thus have a distinct bearing on the nutrition of animals grazing on dried range feed which has been subjected to rain.

The composition of the unleached material and of the residue after leaching of the samples of naturally-dried range feed is given in tables 7 and 8. The residues were not analyzed in this case but have been computed from the analyses of the original and of the extract.

TABLE 7

COMPARISON OF THE PERCENTAGE COMPOSITION OF THE UNLEACHED PORTIONS WITH LEACHED RESIDUES OF NATURALLY-CURED FORAGE; SILICA AND MOISTURE-FREE BASIS

	No. 200, soft chess		No. 212, alfilaria		No. 215, bur clover	
	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue
Crude protein	9.43	8.64	5.74	5.49	15.02	14.62
Nitrogen-free extract	60.44	59.55	53.33	51.41	43.19	41.04
Ether extract	1.69	1.89	2.65	2.88	1.91	2.14
Crude fiber	25.70	28.78	34.68	37.70	33.37	37.31
Silica-free ash	2.74	1.14	3.60	2.52	6.51	4.89
Calcium	0.36	0.28	1.27	1.23	1.26	1.27
Phosphorus	0.27	0.19	0.11	0.07	0.25	0.19
Chlorine	0.22	0.07	0.27	0.11	0.39	0.10

Table 7 shows that the protein and nitrogen-free extract of the residue is lower than that of the original samples, and the ether extract and crude fiber is greater. The most significant change was in the amount of silica-free ash. The calcium content of soft chess was reduced by leaching, but there was no appreciable change in the calcium content of alfilaria and bur clover. In all samples the phosphorus was lowered and the chlorine greatly reduced.

TABLE 8

COMPARISON OF THE PERCENTAGE COMPOSITION OF THE UNLEACHED PORTIONS WITH LEACHED RESIDUES OF NATURALLY-CURED FORAGE (SOXHLET EXTRACTION); SILICA AND MOISTURE-FREE BASIS

	No. 221, soft chess		No. 222, wild oats		No. 226, bur clover		No. 228, alfilaria	
	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue	Unleached sample	Leached residue
Crude protein	6.78	6.65	4.56	4.39	18.23	17.74	5.24	5.23
Nitrogen-free extract	55.29	54.67	59.92	59.50	46.80	44.79	54.91	51.78
Ether extract	2.39	2.52	1.85	2.02	3.63	4.12	2.82	3.21
Crude fiber	31.54	33.21	30.05	32.78	25.71	29.18	28.72	32.65
Silica-free ash	4.00	2.95	3.62	1.31	5.63	4.17	8.31	7.13
Calcium	0.24	0.19	0.29	0.17	1.20	1.23	1.81	1.82
Phosphorus	0.25	0.21	0.13	0.11	0.30	0.25	0.08	0.05

In agreement with the previous experiments, table 8 shows that the greatest change was in the amount of silica-free ash. The phosphorus was lowered in the residues of all the samples; the calcium was reduced in wild oats and soft chess; but the percentage remained practically unchanged in the alfilaria and bur clover. The protein and nitrogen-free extract of the residues were slightly lower than that of the unleached samples, and the ether extract and crude fiber were greater, as was found with the other samples of naturally-cured forage.

DISCUSSION

It was recognized from the beginning that it would be impossible to conduct these experiments so that the leaching would be comparable to that resulting from a given amount of rainfall on the range. The methods which have been used are therefore purely arbitrary and intended only to show the relative losses of the various constituents in order that the reasons for the observed decrease in nutritive value might be more clearly understood.

The results of some of the experiments probably represent nearly complete extractions. Probably the amount extracted was not in excess of that sometimes occurring on the range, when the feed remains saturated for one or two days and is leached by intermittent showers totaling one to three inches or more of rainfall.

The loss of a high percentage of the silica-free ash is significant. Elliot, Orr, and Wood[7] in part II of their investigation on the mineral content of pasture grass in the British Isles concluded that there was no striking difference in the total energy value of good and poor pastures, but that wide differences existed in the proportion of the mineral constituents and that high mineral content was associated with high nutritive value.

Aside from the specific functions of inorganic elements in metabolism, the concentration of mineral salts in the intestinal tract appears to have important functions in the processes of digestion. In regard to this, Orr[8] states: "The ebb and flow of fluid between the lumen of the gut and the blood stream is controlled by the concentration of mineral salts in the intestinal contents and the membrane lining the intestines. An increased amount of mineral salts in the intestinal contents tends to cause a flow of fluid from the blood to the intestines which in extreme cases causes diarrhea." It is a common observation on the ranges during the dry season that the feces of cattle become dry and comparatively hard. There is evidence that the mineral content of the feed may be responsible.

The loss of chlorine together with that of sodium (Le Clerc and Breazeale[5]) undoubtedly accounts for the increase in salt consumption observed in cattle grazing upon forage which had been damaged by rain.

The removal of the most soluble carbohydrates and proteins may leave in the residue the more complex compounds and nutrients which are protected from water and enzyme action by cellulose walls, thus resulting in lower digestibility. The increased fiber content may also have the effect of depressing the digestibility of the other constituents,[9, 10] in addition to being itself more difficult of digestion than other forms of carbohydrate. The reduction of soluble salt and of the soluble carbohydrate may have a marked effect on palatability.

A comparison of the composition of the unleached samples and the residues after leaching does not indicate clearly the extent to which leaching has occurred. A slight increase in one nutrient in the residue might mean a considerable loss in certain other nutrients which would be shown only by an analysis of the extract. An increase of from 3 to 6 per cent in crude fiber for example, was coincident, in these experiments, with losses of from 10 to 20 per cent of total solids.

SUMMARY

The greatest percentage loss caused by leaching was of the constituents of the silica-free ash, which represents that portion of the mineral in the plant which is available to the animal. This loss varied from 25 to 67 per cent in the different samples.

Of the ingredients of the ash which were analyzed, chlorine was lost in greatest amount. The percentage loss amounted to 67 per cent in oat hay and as high as 86 per cent in bur clover. The experiments indicate that practically all of the chlorine may be leached out of dried pastures by excessive rainfall. This is in agreement with the observed salt requirements of stock after feed has been damaged by rain.

In the bur clover and alfilaria samples the percentage of calcium in the forage after leaching was not significantly different from the unleached portion. Phosphorus was distinctly lower in the leached material, particularly in the case of the bur clover leaves and stems. The ratio of calcium to phosphorus in these species thus tends to be widened by leaching. In the grass species a larger percentage of calcium was lost, and the percentage in the residue was lower than in the unleached sample. This may be significant from the standpoint of nutritive value because these dry grasses are in general probably below optimum in calcium. Since the phosphorus is also reduced, the Ca:P ratio remained practically unchanged.

The amount of nitrogen-free extract lost by leaching varied from 6 per cent of the total in a sample of dry bleached soft chess to 35 per cent in bur clover stems and leaves. This loss represents largely the sugars, which are easily digested and which may also influence palatability.

The amount of crude protein lost varied from 1 per cent of the total in oat hay to 16 per cent in bur clover and 18 per cent in soft chess. The loss of protein by leaching from the samples which were cut green and dried was relatively less than that of other constituents, so that there was a higher per cent of protein in the residue than in the original sample. In the naturally-cured samples there was a reduction in the per cent of protein in the leached residue as compared with the original sample. In general the change in percentage of protein does not appear very significant, but there may be a very

significant difference in availability of the residual material as compared with that extracted.

Ether extract is influenced only slightly by leaching. The percentage in the residue is higher than in the original material.

Crude fiber remained entirely in the residue. The decrease in other nutrients caused a very significant increase in the percentage of this material in the dry matter after leaching. An increase of from 3 to 6 per cent in crude fiber was coincident with losses of from 10 to 20 per cent of total solids. The increased fiber content may have a depressing effect upon digestibility of other nutrients in addition to being, itself, difficult of digestion.

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THE RESISTANCE OF VARIETIES AND NEW DWARF RACES OF TOMATO TO CURLY TOP (WESTERN YELLOW BLIGHT OR YELLOWS)[1]

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INTRODUCTION

Certain tomato varieties are known to be resistant to curly top,[3] formerly known as western yellow blight. Curly top is transmitted, in the United States, to beets, tomatoes, and a great variety of other host plants by the leafhopper *Euttetix tenellus* (Baker). As the absence of disease in some previous trials of resistance in tomato varieties was believed to be due to a lack of infective leafhoppers, viruliferous leafhoppers were confined on the plants in some of the experiments reported in this paper. However, trials under natural infestation were thought to be still necessary to test the value of natural resistance as a practical means of control. Accordingly, it was decided to make use of both natural and artificial infestation. An account of trials with natural infestation during three years, 1922–1925, has previously appeared in this journal.[(5)]

[1] Paper No. 234, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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[3] Since the disease is caused by the same virus as curly top of sugar beets, the name curly top, which was first used with the tomato by Carsner and Stahl[(4)] and subsequently by Severin,[(6)] is preferred to 'yellows.'[(8)] The name chosen has the additional advantage of avoiding confusion with yellows of asters, a disease caused by a different virus, which is transmissible to the tomato.

METHODS

The investigation was carried on at the Citrus Experiment Station, Riverside, California, and at the Cotton Field Station, United States Department of Agriculture, Shafter, California.[4] At Shafter in 1926–1929 and at Riverside in 1926 the plants were started in cold frames, but at Riverside in 1927–1930 they were started in paper pots in a heated greenhouse. Transplanting to the field was done late in April or in the first fortnight of May, which is about the usual time of planting when the crop is intended for the cannery. Santa Clara Canner, Stone, and Norton, important canning varieties susceptible to yellows, were planted as checks. Varieties which were less susceptible than the check varieties when exposed only to natural infestation in the field by the leafhoppers are termed resistant. In recording the condition of the plants, the first definite symptoms of curly top were denoted by *E*, signifying an early stage of the disease, and fully developed symptoms, especially yellowing, by *Y*. Only plants showing definite symptoms of curly top were counted as affected. The probable error of the percentage affected (tables 1 to 3) was obtained from the formula:

$$\text{P. E.} = 0.67 \times \sqrt{p \times q \div n}$$

where p is the observed percentage affected, and $q = 100 - p$. Since it is known that the standard deviation of the observed percentages may far exceed that of simple sampling[(5)] the probable error must be used with reserve, especially where p approaches its limiting values.

NATURAL INFESTATION

The results of the trials with plants exposed only to natural infestation are shown in tables 1 to 3. At Shafter in 1926 an extremely severe epidemic of curly top occurred early in the summer. As in some previous trials there, no variety had sufficient resistance to survive, and on June 23 only 4 plants out of over 800 remained healthy. Progeny tests of these plants indicate that they were not exceptionally resistant. At Shafter in 1928 and at Riverside in 1927 to 1929 less than 5 per cent of curly top occurred. At Shafter in 1927 and 1929 and at Riverside in 1926 moderate epidemics occurred. Selected

[4] The writer is especially indebted to Dr. E. Carsner, and Mr. M. Shapovalov, Senior Pathologists, United States Department of Agriculture, for many helpful suggestions.

TABLE 1

TOMATO CURLY TOP AT RIVERSIDE, CALIFORNIA, IN 1926; NATURAL INFESTATION*

	Number recorded	Per cent affected, June 28	Per cent affected, whole season	Probable error of sampling of percentage affected, whole season
Santa Clara Canner	39	23	64	5.2
Dwarf Aristocrat	86	5	34	3.4
Philippine Wild	39	18	56	5.3
Selected line from:				
Stone	41	29	66	5.0
Santa Clara Canner, series 73-1†	193	19	62	2 3
Santa Clara Canner, various	248	20	62	2 1
Dwarf Aristocrat × Red Pear F_3, dwarf	254	9	50	2 1
Dwarf Champion × Santa Clara Canner F_3, dwarf	151	9	53	2 7
Dwarf Aristocrat × Santa Clara Canner F_3, dwarf	172	5	38	2.5
(Dwarf Aristocrat × Red Pear, F_1)×(Dwarf Champion × Santa Clara Canner, F_1), standard	27	26	59	6 3
(Dwarf Aristocrat × Red Pear F_1) × (Dwarf Champion × Santa Clara Canner F_1), dwarf	17	6	30	7.4

* The following races were tested on a small scale and found to be susceptible: dwarf yellow pear; standard peach pear; dwarf compound; Manx Marvel X Santa Clara Canner F_1.

† Compare Hilgardia 2:61, *table 5*, 1926.

TABLE 2

CURLY TOP AT SHAFTER, CALIFORNIA, IN 1927; NATURAL INFESTATION

	Sown February 25 Transplanted May 14				Sown Feb. 25 Transplanted June 2		Sown May 14 Transplanted July 6	
	Number recorded	Per cent affected June 16	Per cent affected whole season	Probable error of sampling of percentage affected whole season	Number recorded	Per cent affected whole season	Number recorded	Per cent affected whole season
Dwarf Aristocrat	63	44	52	4 2	20	5	148	1
Dwarf Yellow Pear	22	32	50	7.1	29	21	....	....
Red Pear	47	32	53	4 9	51	37	....	....
Yaqui Valley Wild*	29	14	48	6 2	....	....	....	....
Marglobe	17	12	18	6 2	7	29	....	....
Selected line from:								
Stone	27	33	48	6 4	....	....	....	....
Merced Stone	47	43	66	4.6	17	30	....	....
Santa Clara Canner, series 376	71	27	56	4.0	9	22	....	....
Santa Clara Canner, series 73-1	80	41	65	3.6	....	....	....	....
Dwarf Champion	5	20	20	......	10	10	....	....
Dwarf Aristocrat × Santa Clara Canner, F_4, dwarf	122	33	53	3.0	67	16	359	1
Red Pear × Dwarf, dwarf	20	40	60	7.4	....	....	...	....
Dwarf Aristocrat × Red Pear F_3, dwarf	92	25	38	3 4	48	19	....	....
Dwarf Aristocrat × Red Pear F_4, dwarf	68	12	38	3.9	29	7	....	....

* Collected by Mr. W. W. Mackie, Associate Agronomist, University of California.

lines from Santa Clara Canner, which were previously believed to be resistant, were as much affected as the parent variety (table 1) but Dwarf Aristocrat and most of the progenies of dwarf habit derived from crosses between Dwarf Aristocrat or Dwarf Champion and Santa Clara Canner were resistant. At Shafter in 1929, the difference between the per cent affected in the dwarf hybrid progeny of 2–7–1–3 and in Santa Clara Canner was 8.2 times the probable error (table 3). No variety of standard (nondwarf) habit was resistant except Red Pear, which in a single test at Shafter (table 2) seemed as resistant as Dwarf Aristocrat. Of the progenies of dwarf habit from Dwarf Aristocrat × Red Pear, none was clearly more resistant than Dwarf Aristocrat. Hitherto all dwarf varieties tested have appeared to be resistant, but a few dwarf progenies appear to be susceptible, in particular the F_6 from 2–7–1–5, which differed from Dwarf Aristocrat by 4.3 times the probable error (table 3).

TABLE 3

CURLY TOP AT SHAFTER, CALIFORNIA, IN 1929; NATURAL INFESTATION

	Pedigree number	Transplanted May 8				Transplanted May 14	
		Number recorded	Per cent affected May 31	Per cent affected whole season	Probable error of sampling of percentage affected whole season	Number recorded	Per cent affected whole season
Dwarf Aristocrat		200	24	71	2 1	....	....
Santa Clara Canner		83	46	94	1.7	16	100
Parana (Argentine)		61	26	93	2 2	32	81
Peru Wild*		46	24	96	1 9	....	....
Selected line from:							
Gigante liscio		90	29	94	1.7	12	92
Stone, series 52-1-1		367	25	88	1.1	32	75
Dwarf Aristocrat × Santa Clara Canner, F_6, dwarf	2-7-1-1	99	10	70	3 1	....	....
	2-7-1-3	100	14	64	3 2	....	....
	2-7-1-4	93	11	74	3.0	....	....
	2-7-1-5	97	15	85	2.4	....	...
	1-2-1-1	91	21	79	2.8	12	83
	1-2-1-3	52	6	62	4.5	....	....
	1-7-1-1	66	17	85	2.9	....	....
Dwarf Aristocrat × Red Pear, F_5, dwarf		93	10	70	3.2	10	40

* Collected by Mr. O. F. Cook, U. S. Dept. of Agriculture.

The influence of time of planting on the intensity of curly top was well illustrated at Shafter in 1927 (table 2). In Dwarf Aristocrat and a dwarf hybrid sown on February 25 and transplanted on May 14 over 50 per cent were affected, but in the same varieties sown on May 14 and transplanted on July 6, only 1 per cent.

It is evident from these and from previous trials that certain dwarf varieties and hybrids and also Red Pear are slightly resistant to curly top; resistance in the dwarfs is recessive and is due to the *d* (dwarf) gene or to a gene or genes closely linked with it. Although the resistance of the dwarf varieties is not sufficient to withstand very severe attacks of curly top, it will help to moderate the loss due to this disease. The percentage losses of plants due to curly top in field trials at Riverside in 1923 to 1926 and 1929 in the susceptible varieties, Stone, Norton, and Santa Clara Canner were 2, 52, 44, 62, 4; the corresponding losses in resistant dwarf varieties were 0, 32, 21, 34, 3. At Shafter in 1923 to 1929 the losses in the susceptible varieties were 14, 100, 100, 99, 58, 9, 94, and in the resistant varieties 8, 99, 99, 100, 50, 6, 72. In five epidemics of moderate severity the mean loss of stand due to curly top was 62 per cent in susceptible varieties as against 42 per cent in resistant dwarf varieties.

In order to make practical use of resistance, the attempt has been made to breed dwarf varieties resistant to curly top and at least equal in other respects to the standard varieties now in cultivation. Some of the F_6 progenies of dwarf habit obtained from Dwarf Aristocrat × Santa Clara Canner, such as those from 2–7–1–3 and 1–2–1–3 (table 3) seem to combine resistance equal to that of the dwarf parent with large size and good quality of fruit for canning, sufficient vine to shade the fruit, and earlier maturity than Santa Clara Canner. These dwarf varieties may be planted more closely than standards and may prove useful in sections where curly top is prevalent.

ARTIFICIAL INFESTATION

Artificial infestation consisted in confining a number of the leafhoppers, *Eutettix tenellus* (Baker), for two days in a celluloid cage attached to the tip of a tomato shoot. The cage was of the type used by Shapovalov[(9)] and consisted of a cylinder of celluloid 10 cm long and 6.5 cm in diameter with cheesecloth ends. As a rule one cage was attached to a plant. For maintaining and increasing the supply of leafhoppers, sugar beets were used as host plants. For two days preceding their use the leafhoppers were kept on severely diseased leaves of susceptible beets. If, after two days' confinement on tomato, one or more insects survived, as was usually the case, the plant was counted as having been artificially infested or as 'treated.' In many cases tomato plants which had been artificially infested did not become diseased, although they were certainly not all genetically

resistant. Apparently the virus was either not introduced into them at all or not in such a manner as to cause disease. At Riverside where the artificial infestations were made, in all four seasons, 1927 to 1930, not more than 5 per cent of the plants became diseased through natural infestation.

Shapovalov[7] has observed that young tomato plants are more susceptible to curly top than larger and older plants, and Shapovalov and Beecher[10] found that the development of curly top in infected plants is influenced by environmental conditions. Carsner and Lackey[3] reported that, with curly top of sugar beets, the chance of infection and the incubation period are influenced by the number of leafhoppers per plant. Evidence is presented below (p. 40) that in the tomato similar effects are produced by variation in the number of leafhoppers. Consequently, in judging resistance, only plants of the same age infested on the same days and exposed to the same number of leafhoppers are directly comparable.

In 1928 about 850 plants were artificially infested, including progenies of plants which had survived natural or artificial infestation in the previous year and a rather large number or varieties not previously tested. The results are shown in tables 4 and 5. The first infestation was made 21 days after transplanting and other infestations as late as August 15 when the vines had probably attained their maximum size. As a rule, 5 leafhoppers were used to a plant. The mean percentage affected from infestations with 5 leafhoppers on June 7, June 13, and June 27 (table 4) was less in Dwarf Aristocrat, the dwarf hybrids, and Red Pear than in Santa Clara Canner. A few plants of some of these varieties subjected to 5 hoppers on July 20 (table 4) and to repeated infestations (table 5) behaved in a similar manner. The progenies of series 73–1 seemed to be fairly susceptible under artificial infestation just as those of closely similar origin were susceptible under natural infestation (table 2). On the other hand, after infestation with 15 hoppers on August 1 (table 4), the proportion affected in Santa Clara Canner was less than in the dwarf races. The progeny of series 52–1–1 seemed to be resistant when artificially infested, although under natural infestation (table 3) the progenies of its derivatives were clearly susceptible. On the whole, the results of the artificial infestation in 1928, while somewhat conflicting, indicate that varieties resistant in natural infestation are also resistant when artificially infested.

TABLE 4

CURLY TOP AT RIVERSIDE, CALIFORNIA, IN 1928*

Date of artificial infestation is followed by number of insects used, in parentheses.

	June 7 (3)		June 7 (5)		June 13 (5)		June 27 (5)		June 30 (5)		July 20 (5)		July 25 (10)		Aug. 1 (15)		Mean per cent affected of plants treated June 7, 13, and 27	Mean per cent affected of plants treated June 7 and June 30
	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected	Number treated	Number affected		
Santa Clara Canner	....	....	6	5	16	10	12	6	....	....	10	5	....	....	6	1	65	....
Dwarf Aristocrat and Dwarf Champion	6	1	9	6	16	7	8	4	8	1	13	5	....	....	14	7	47	....
Red Pear	....	..	6	5	14	4	15	4	....	....	....	....	....	....	10	3	46	....
Selected line from:																		
Stone, series 52-1-1	....	....	5	1	14	4	11	1	....	....	10	5	....	....	7	2	19	....
Stone, various	....	....	11	9	....	....	....	....	16	4	....	....	....	....	....	....	....	53
Marglobe	...	....	4	2	....	....	....	....	8	3	....	....	10	4	....	....	....	44
Santa Clara Canner, series 73-1	....	....	7	5	12	5	10	6	8	2	....	....	....	....	....	....	58	....
Santa Clara Canner, series 400-4	....	....	4	2	4	2	....	....	10	5	....	....	....	....	....	....	....	50
Dwarf Aristocrat × Santa Clara Canner, F_4, dwarf	20	10	27	17	46	17	40	5	18	4	11	2	....	....	13	8	35	....
Dwarf Aristocrat × Red Pear, F_4, dwarf	8	3	26	15	30	13	27	8	20	6	10	1	....	....	12	5	42	....

* The following varieties were also artificially infested and were found to be susceptible: Précoce Trophy, Nano e frutto grossissimo, Re Umberto, Meraviglia, di S. Marzano, Vittorio Emanuele, Dwarf Giant.

On June 2, 1930, 16 days after transplanting into the field, plants of a resistant dwarf race and of a susceptible variety were infested with 5 insects each, and other plants of similar varieties with 10 insects. When 5 leafhoppers were used 17 out of 24 plants of a resistant dwarf race and 20 out of 25 plants of a susceptible variety became diseased. When 10 insects were used, 22 out of 27 plants of a resistant dwarf race and all 27 plants of a susceptible variety became diseased. The proportion affected therefore was slightly less in the resistant races whether 5 or 10 insects were used for infestation.

TABLE 5

CURLY TOP AT RIVERSIDE, CALIFORNIA, IN 1928; REPEATED ARTIFICIAL INFESTATION

Date of infestation is followed by number of insects used, in parentheses.

	June 7 (3 to 5) or June 13 (5) or June 18 (5) and July 20 (10) or July 25 (10)		June 27 (5) and August 15 (25)	
	Number treated	Per cent affected	Number treated	Number affected
Santa Clara Canner	6	83	6	1
Dwarf Aristocrat	15	47	3	2
Dwarf Aristocrat, regenerated plant	1	100	....	....
Red Pear	10	40	....	....
Selected line from:				
Stone, series 52-1-1	14	64	8	4
Stone, regenerated plant	1	100	....	....
Santa Clara Canner, series 73-1	7	86	....	....
Dwarf Aristocrat × Santa Clara Canner, F_4, dwarf	31	52	16	4
Dwarf Aristocrat × Red Pear, F_4, dwarf	29	59	....	....

Very similar results were obtained from artificial infestation with 5 leafhoppers per plant on May 20, 1931, 20 days after transplanting to the field. On July 27, in a resistant dwarf race 79 per cent of the 56 plants treated were affected and in Norton 88 per cent of the 57 plants treated. In Grape Cluster, a small-fruited variety of standard habit, 69 per cent of the 32 plants treated were affected which indicates that it is at least as resistant as the dwarf race.

In 1927 artificial infestation was begun on July 18, when the plants were about six weeks older than those treated on June 5, 1928. The results are shown in table 6. As in the artificial infestations in June, 1928, as a rule, 5 leafhoppers were used per plant.

In 1929 over 1,000 plants were treated (table 7), beginning on May 25 when the plants were approximately of the same age as the youngest plants treated in 1928. As a rule in 1929 a greater number

of plants of a variety received the same treatment and more leafhoppers were applied per plant than in 1927 or 1928. Tables 6 and 7 show that both in 1927 and 1929 Dwarf Aristocrat and practically all the dwarf hybrids and perhaps also Red Pear were as much affected with curly top as susceptible varieties such as Santa Clara Canner and the progenies of series 52–1–1. Simple trisomic (triplo-A) dwarf plants containing an extra *d* (dwarf) gene were slightly less affected than diploids in the same F_2 population (table 7), but no variety was clearly better able to survive artificial infestation than the susceptible checks. In 1929 some plants which survived the first infestation and were thought to be resistant were reinfested, but all became diseased.

TABLE 6

CURLY TOP AT RIVERSIDE, CALIFORNIA, IN 1927

Five leafhoppers were used per plant.

	Date of transplanting	Date of artificial infestation			
		July 20, 22, 24, and 26		July 28, 29, 30, 31, and August 1	
		Number treated	Per cent affected	Number treated	Per cent affected
Dwarf Aristocrat	May 20 and 25	40	78	11	36
Red Pear	May 20	....	....	14	50
Selected line from:					
Stone	May 25	...	....	10*	30
Morse's Santa Clara Canner	May 20	20	45	5	80
Selected line from:					
Santa Clara Canner series 73-1	May 20	....	...	10†	30
Dwarf Aristocrat × Red Pear, F_3, dwarf	May 20	20	60	15	33
Dwarf Aristocrat × Red Pear, F_4, dwarf	June 6	....	....	14*	50
Dwarf Aristocrat × Santa Clara Canner, F_4, dwarf	May 25	20	85	1	100

* 4 leafhoppers per plant. † 2 leafhoppers per plant.

Apparently when varieties and races known to be resistant under natural infestation were artificially infested in 1928, 1930 and 1931 they showed resistance, but in 1927 and 1929 they failed to do so and behaved like susceptible varieties.

As a smaller number of insects per plant was used in artificial infestations in 1928 than in 1929, it seemed probable that these differences in varietal response might be due to variations in the number of leafhoppers used. But in 1930 some resistance was evident even in young plants of the dwarf races whether 5 or 10 leafhoppers were used for infestation. It appears, therefore, that variations in the number

of insects used do not account for the differences in varietal response in 1928, 1930 and 1931 compared with 1927 and 1929. Moreover, in 1927 only 5 leafhoppers per plant were used and the plants were relatively old when treated, yet the resistant varieties were as much affected as the susceptible checks. Shapovalov and Beecher[10] have shown that climatic conditions, especially the intensity of light, influence the development of symptoms of curly top in infected plants. Possibly the climatic conditions were less favorable for curly top in 1928, 1930 and 1931 and so resistance was evident, whereas in 1927 and 1929 the conditions were more favorable for the disease and resistance was obscured.

Resistance in these tomato varieties is weak and evidently is overcome if the number of viruliferous leafhoppers is sufficiently large and other conditions are favorable to the disease. The sequence of symptoms, namely, cessation of growth, progressive yellowing, and death is the same in the resistant and susceptible varieties, and, as will appear (p. 40), there is very little difference in the incubation periods. Recovery occurs with nearly the same frequency in resistant and susceptible varieties. Moreover, Shapovalov and Jones[11] found that the chemical changes in plants affected with curly top were the same in resistant dwarf and in susceptible strains.

The resistance of sugar beets to curly top which was found in certain selected lines by Carsner,[1] is evidently much greater than in the tomato. Beet plants of the highly resistant strains generally show mild symptoms and plants of less resistant strains more pronounced symptoms of the disease. According to Carsner and Lackey[2] attenuation of the virus occurs in the most resistant strains. Evidently these strains of beets are resistant not only to infection, but to the virus after they become infected. In beets resistance is strong and tolerance of the virus is a main factor, but in tomatoes resistance is weak and probably due to a tendency to escape infection. The dwarf varieties of tomato have a small, darker green vine, more rigid foliage, and a more compact habit of growth than susceptible standard varieties. Red Pear, however, which is resistant, is standard in habit, although distinct in vine from other standard varieties. Apparently these varieties offer some slight mechanical or other barrier to infection, which is overcome when viruliferous leafhoppers feed on them and climatic conditions are favorable to the disease.

TABLE 7

CURLY TOP AT RIVERSIDE, CALIFORNIA, IN 1929*

Date of artificial infestation is followed by number of insects used, in parentheses.

	Date of transplanting	May 25 (5)		May 25 (10)		May 29 (5)		May 29 (10)		June 7 (10)		June 12 (10)		June 12 (20)		June 21 (20)	
		Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected	Number treated	Per cent affected
Dwarf Aristocrat	April 30	....	....	....	....	25	40	....	....	25	28	12	67	17	47	....	....
Santa Clara Canner	April 30	25	64	25	48†	....	....	....	....	12	33	....	....	....	....	....	....
Selected line from:																	
Stone, series 52-1-1	April 30	25	40	....	....	25	60	25	60	8	62	....	....	....	....	....	....
Santa Clara Canner, series 400-4	April 30	11	36	....	....	25	72	....	....	....	....	....	....	....	....	....	....
Dwarf Aristocrat × Santa Clara Canner, F_5, dwarf	April 30	50	64	25	44†	25	64	27	48	33	55	22	77	....	....	10	100
Dwarf Aristocrat × Red Pear, F_4, dwarf	April 30	25	36	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Dwarf Aristocrat	May 13	....	....	....	....	....	....	....	....	25	24	....	....	....	....	....	....
Selected line from:																	
Stone, series 52-1-1	May 13	....	....	....	....	....	....	....	....	18	39	....	....	....	....	....	....
Dwarf Aristocrat × Santa Clara Canner, F_6, dwarf	May 13	....	....	....	....	....	....	....	....	24	62	....	....	....	....	....	....
Dwarf Aristocrat × Red Pear:																	
Diploid, dwarf	May 13	....	....	....	....	....	....	24	62	....	....	....	....	....	....	....	....
Triplo-A, dwarf	May 13	....	....	....	....	....	....	25	40	....	....	....	....	....	....	....	....

* The following varieties were also artificially infested and found to be susceptible: Institute of Applied Botany, U.S.S.R., Nos. 748 and 759 (from Peru, collected by Dr. Juseptschuk); F. P. I. Nos. 55575 and 55841; Uitenhage (South Africa) Wild; *Solanum tomatillo* (Red Currant); Gigante liscio; *S. Humboldtii* (Yellow Cherry).

† Up to June 18.

RECOVERY

Recovery of plants even from an advanced stage of disease sometimes occurs, especially in the late summer and fall. The change is usually accomplished by a process of regeneration in which new shoots grow out from the leaf axils, so that a healthy plant is reproduced, which may even yield a crop of fruit. Of the 400 plants which became diseased owing to artificial infestation in 1928, over 11 per cent recovered. The percentage of plants which recovered was slightly greater in the resistant than in the susceptible varieties, but the difference was not significant. A few plants raised from seed of fruits produced by regenerated plants were artificially infested, but they seemed to be as susceptible as seedlings from healthy parents of the same stock. One plant which regained its normal color after reaching an advanced stage of curly top was artificially infested with 80 leafhoppers. It again developed symptoms of curly top and did not recover. The new shoots produced in the regeneration of affected plants often develop symptoms of curly top. This was especially common at Riverside in 1929. Evidently a tomato plant which recovers is not rendered immune, and probably it is not more resistant than a plant which has not previously been affected.

INCUBATION PERIOD

The incubation period in the plant or the length of time between the removal of the hoppers and the appearance of symptoms, in 1928 and 1929, is shown in tables 8 and 9. The plants were examined at intervals of about a week throughout the period from May to August in which most cases of disease occurred, and at longer intervals during September and October. The incubation period in tables 8 and 9 is therefore approximately correct to the nearest week. Many of the minor fluctuations in the frequency distribution are due to irregularities in the interval between observations. The incubation period was very variable, however, especially after artificial infestations made late in the season when the plants were large. In 1930 the mean incubation period in 49 young plants infested on June 2 with 5 leafhoppers was 20 days and in 54 similar plants treated on the same date with 10 leafhoppers 19 days, but in 14 of the 59 cases of curly top following artificial infestation on June 27 and 30, 1928 (table 8), the

TABLE 8

FREQUENCY DISTRIBUTION OF INCUBATION PERIOD OR NUMBER OF WEEKS FROM ARTIFICIAL INFESTATION TO APPEARANCE OF SYMPTOMS OF CURLY TOP AT RIVERSIDE IN 1928

	Date of infestation	Number of leaf-hoppers used	Incubation period in weeks from date of last infestation to early stage of curly top*														Mean incubation period in weeks to E stage of curly top	Mean incubation period in weeks to Y stage of curly top
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
All varieties	June 7	5 or 3	0	3	15	19	13	4	2	2	0	0	0	1	0	—	4	5
	June 13	5	2	2	17	14	10	4	0	0	0	0	0	0	—	0	4	5
	June 27	5	0	2	4	11	4	2	1	3	1	0	—	1	—	1	5	6
	June 30	5	—	4	—	12	5	1	3	2	—	1	—	1	—	1	5	6
	July 20	5	0	0	0	0	—	5	6	—	2	—	0	—	0	....	7	8
	June 7 or 13 and July 20	5 10	5	2	2	4	—	16	6	—	2	—	1	—	0	....	5	7
	July 25	10	1	0	0	1	5	5	—	5	—	1	—	3	...	....	7	8
	August 1	15	0	0	0	0	5	—	10	—	5	—	1	....	....	....	7	8
	June 7 or 13 and July 25	5 10	0	0	0	0	2	7	—	5	—	0	—	0	....	....	7	9
Resistant varieties	June 7	5 or 3	0	1	13	13	10	3	2	1	0	0	0	0	0	—	4	5
	June 13	5	0	0	11	10	7	3	0	0	0	0	0	0	—	0	4	5
	June 27	5	0	1	2	5	2	2	1	3	1	0	—	0	—	1	6	7
	July 20	5	0	0	0	0	—	4	1	—	1	—	0	—	0	....	7	8
Susceptible varieties	June 7	5 or 3	0	1	1	4	1	1	0	0	0	0	0	0	0	—	4	5
	June 13	5	0	2	7	4	2	1	0	0	0	0	0	0	—	0	4	4
	June 27	5	0	1	2	6	2	0	0	0	0	0	—	1	—	0	4	6
	July 20	5	0	0	0	0	—	1	5	—	1	—	0	—	0	....	7	9

* Dash (—) indicates that no observation was made during this period.

incubation period was 7 weeks or more. During the period August 20 to October 17, when these cases occurred, among 438 plants not artificially infested 8 at the most became affected, so that probably not more than 1 or 2 of those 14 cases were due to natural infestation. Evidence of a long incubation period is also given by the frequency distribution for plants first treated June 7 or 13, 1928, and again treated July 20 (table 8). Seven of the 38 affected plants showed early symptoms 1 to 2 weeks after reinfestation, only two plants 3 weeks after and the majority 4 to 7 weeks after. The discontinuity of this distribution suggests that the earliest cases were due to the *first infestation* on June 7 or 13, so that the incubation period for these cases was at least 6 weeks.

On account of the difference in color and texture of the leaves it is diffcult to determine when plants of dwarf and standard varieties have reached precisely the same stage of disease. The incubation period in 1928 and 1929 in groups of varieties resistant and susceptible to natural infestation is also shown in tables 8 and 9. In 1930, the incubation period in 16 plants of a resistant dwarf race treated on June 2 with 5 leafhoppers was 23 days and in 21 plants of a susceptible variety it was 17 days; in 21 plants of another resistant dwarf race treated on June 2 with 10 insects it was 19 days and in 27 plants of a susceptible variety it was 18 days. In most cases the mean incubation period of both stages of curly top was longer in the resistant than in the susceptible group, but the differences may not be significant. The fact that the incubation period in resistant and susceptible varieties was so similar is some evidence of a negative kind that, in this tomato material, resistance to the virus is unimportant. In the present work no very young plants were artificially infested, but in 1928 (table 8) plants of several varieties treated June 7 had a shorter incubation period than plants treated June 30, in accordance with expectation. In 1929 (table 9), when the number of leafhoppers caged on plants of the same age varied, as a rule the incubation period was shorter when the number of leafhoppers was greater.

TABLE 9

FREQUENCY DISTRIBUTION OF INCUBATION PERIOD OR NUMBER OF WEEKS FROM ARTIFICIAL INFESTATION TO APPEARANCE OF SYMPTOMS OF CURLY TOP AT RIVERSIDE IN 1929

	Date of infestation	Number of leaf-hoppers used	Incubation period in weeks from date of last infestation to appearance of early symptoms												Mean incubation period in weeks to E stage of curly top	Mean incubation period in weeks to Y stage of curly top
			1	2	3	4	5	6	7	8	9	10	11	12		
All varieties	May 25	5	....	...	24	2	11	3	5	1	1	5	—*	0	5	6
	May 25	10	....	...	17	2	3	3	1	0	0	0	—	0	4	5
	May 29	5	...	0	4	17	11	—	3	6	5	2	2	—	6	6
	May 29	10	...	0	5	16	32	—	3	5	3	1	0	—	5	6
	June 7	10	0	1	31	5	—	9	4	4	1	0	—	4	5	7
	June 7	20	0	1	6	1	—	3	0	0	0	0	—	0	4	6
	June 12	10	0	0	13	—	3	1	1	1	0	—	1	—	4	7
	June 12	20	0	0	7	—	0	1	1	1	0	—	1	—	5	6
	May 25	5 or 10	1	0	—	5	0	0	1	0	—	0	—	—	4	5
	and June 21	20														
	May 25	10	2	3	2	1	0	—	0	0	—	—	0	—	2	6
	June 21	20														
	and June 29	40														
	June 21	5	0	1	—	3	2	1	0	0	—	0	—	—	4	8
	June 21	20	0	0	—	10	3	1	0	1	—	0	—	—	5	6
Resistant varieties	May 25	5	....	....	16	2	5	2	4	1	0	4	—	0	5	6
	May 25	10	....	....	8	1	1	0	0	0	0	0	—	0	3	6
	May 29	5	....	0	3	5	3	—	0	3	1	1	2	—	7	6
	May 29	10	....	0	2	11	22	—	3	2	3	1	0	—	5	7
	June 7	10	....	0	25	2	—	7	1	4	0	0	—	2	5	7
Susceptible varieties	May 25	5	....	....	8	0	3	1	1	0	1	0	—	0	4	5
	May 25	10	....	...	8	0	2	2	0	0	0	0	—	0	4	5
	May 29	5	....	0	1	3	2	—	1	0	0	0	0	—	4	5
	May 29	10	....	0	0	5	9	—	0	3	0	0	0	—	5	6
	June 7	10	....	1	6	3	—	2	3	0	1	0	—	2	5	7

* Dash (—) indicates that no observation was made during this period.

INFLUENCE OF THE NUMBER OF LEAFHOPPERS

The data in table 10 show the effect of the number of leafhoppers used in artificial infestation on the probability of infection. In seven of the nine tests with different tomato varieties in 1928, 1929, and 1930, the percentage affected with curly top increased with the number of leafhoppers used. In only one test the opposite was the case, and in one there was no difference. The conclusion seems justified that in tomatoes, as in sugar beets,[3] the probability of infection is partly determined by the number of infesting leafhoppers. Whether this effect is due to variations in the quantity of inoculum introduced or in the infectivity of the individual leafhoppers or to some other cause, is at present uncertain.

TABLE 10

THE EFFECT OF VARIATIONS IN THE NUMBER OF LEAFHOPPERS USED ON THE INCIDENCE OF CURLY TOP AT RIVERSIDE, CALIFORNIA. IN EACH COMPARATIVE TEST THE SAME VARIETY OF TOMATO WAS USED

1928				1929				1930			
Date of infestation	Number of leafhoppers used	Number treated	Per cent affected	Date of infestation	Number of leafhoppers used	Number treated	Per cent affected	Date of infestation	Number of leafhoppers used	Number treated	Per cent affected
June 7	3	34	38	May 29	5	25	60	June 2	5	49	75
June 7	5	34	56	May 29	10	25	60	June 2	10	54	89
June 30	5	13	38	June 7	10	57	25				
June 30	15	13	46	June 7	20	15	87				
July 2	5	11	27	June 12	10	12	67				
July 2	10	9	44	June 12	20	17	47				
July 25	5	9	0	June 21	5	10	70				
July 25	25	8	50	June 21	20	10	100				

SUMMARY

As in previous trials, some tomato varieties of dwarf habit and also Red Pear, a variety of standard habit, proved to be resistant to curly top when exposed to natural infestation by leafhoppers (*Eutettix tenellus* Baker). In epidemics of moderate severity, the mean loss of plants from curly top in five trials in four seasons, at two places, was 42 per cent in resistant dwarf varieties and 62 per cent in the susceptible varieties—Santa Clara Canner, Norton, and Stone. In epidemics of extreme severity all varieties became nearly 100 per cent diseased. Attempts to isolate resistant lines from commercial varieties of standard habit have failed. No increase in resistance has been obtained by crossing a resistant dwarf with Red Pear. By hybridization, improved dwarf varieties have been obtained which may prove useful for localities where curly top is a serious menace.

When resistant and susceptible varieties were artificially infested, the results were variable. In three seasons the resistance of the dwarf races was evident as in natural epidemics of moderate intensity, but in the other two seasons no resistance was apparent, although a considerable proportion of the plants of resistant and susceptible varieties did not become diseased. Consequently artificial infestation, at least with small numbers of plants, has not proved reliable as a means of testing the resistance of tomato varieties under natural infestation. The variation in the response under artificial infestation was probably not due to variations in the number of insects used but may have been due to differences in climatic conditions.

The resistance is weak and seems to be due not so much to tolerance of the virus as to a tendency to escape infection. The chance of infection is influenced by the number of leafhoppers used in artificial infestation. The incubation period of the disease after artificial infestation of plants not less than 3 weeks after transplanting varied from 2 to at least 7 weeks. No significant difference was found in the length of the incubation period or in the frequency of recovery in resistant and susceptible varieties, and resistance was not increased in plants which had recovered or in their progeny.

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DISTRIBUTION OF SOLID MATTER IN THICK AND THIN EGG WHITE[1]

W. F. HOLST[2] AND H. J. ALMQUIST[3]

Stored eggs kept under optimum conditions of temperature and humidity and free from molds and putrefactive bacteria may, nevertheless, exhibit tendencies toward undesirable changes which cause the eggs to lose much of their original appearance and attractiveness.

One of the most prominent of these changes is the slow liquefaction of the firm, jelly-like white. As a result of this liquefaction the egg white appears watery. This condition is found very objectionable in the market egg and frequently results in a lowering of grade and price of the egg with a corresponding loss to the owner.

Up to the present time investigations of egg white have not differentiated between the thick and the thin varieties in kind studied or in results obtained. Accordingly, there has existed no experimental evidence which would serve as a basis for an explanation of the progressive liquefaction often encountered in stored eggs.

The results of investigation of thick and thin white can hardly be considered comparable until the amount of dry matter present in each of these substances is known and any variation in this dry matter is taken into account. To establish a basis of comparison of thick and thin white, as a first step in studies on watery whites, the distribution of dry matter in thick and thin white, and its possible variability in different eggs were investigated.

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METHODS

The method of determining total solids was that recommended by Hertwig (1925): A sample of 2 grams of liquid white was weighed into a covered aluminum dish which had previously been dried at 127–133° C, allowed to cool in a desiccator, and weighed soon after attaining room temperature. The dish was uncovered and, with contents and cover, dried in the oven at 127–133° C for one hour. The dish was then covered, transferred to desiccator to cool to room temperature, and weighed. The method has proved satisfactory and capable of very close checks.

Refractive index was measured with the Spencer Refractometer, Abbe type. The investigation included normal eggs up to 40 days of age from more than 30 birds. The eggs were stored under room conditions.

RESULTS

Without exception, the refractive index and total solids of thin white were found identical within experimental limits with those of thick white from the same egg in eggs more than 1 day old. Occasional small and random differences appeared in eggs less than 1 day old.

TABLE 1

Hen	Age of egg, days	White	Solids	Refractive index 20°C
D 512	1	thin	11.24	1.3551
		thick	11.26	1.3550
	2	thin	11.76	1.3559
		thick	11.72	1.3559
	3	thin	11.80	1.3561
		thick	11.84	1.3562
D 511	2	thin	12.25	1.3569
		thick	12.22	1.3568
	20	thin	13.08	1.3582
		thick	13.12	1.3582
	30	thin	14.71	1.3610
		thick	14.74	1.3610
	39	thin	15.26	1.3620
		thick	15.22	1.3620

Representative data are included in detail in table 1; all data secured are presented graphically in figure 1. The values for percentage solids in thick and thin white from the same egg have been plotted against the corresponding average refractive index, since these measurements seem to be the same for each kind of white. The relation is

practically linear regardless of the variation in age or composition of the white.

In the fresh eggs studied the egg-white solids were found to vary chiefly in the range 10.7 to 12.9 per cent, with a corresponding refractive index variation of 1.3540 to 1.3580. A few extremes were found outside of these limits, values as high as 13.5 per cent and as low as 9.6 per cent being observed.

Eggs from the same hen showed much less variation and in many cases a high degree of uniformity. The percentage of solids in the white of stored eggs tended to increase in proportion to an increase in refractive index; these changes were in general more pronounced in the eggs which showed greater shrinkage.

DISCUSSION

The refractive index shows a steady increase with age but maintains throughout the same relation to total solids, as demonstrated in figure 1. The increase in concentration of solids is undoubtedly due to the disappearance of water from the white. This loss may take place in two ways, namely, by escape of water vapor through the

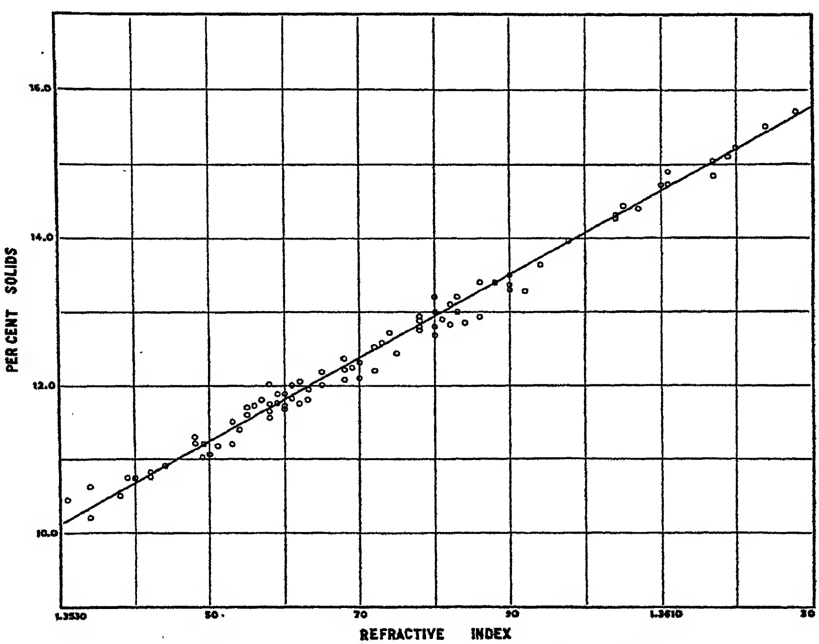

Fig. 1. The relation of the refractive index of egg white to its solids concentration.

shell and by diffusion of liquid water into the yolk. In order that equality of refractive index and total solids may persist, there must exist, between thick and thin white, a rapid equilibrium with respect to water. The loss of carbon dioxide and the gradual disappearance of thick white seem to have no bearing on this relation.

It is believed that the lack of complete agreement between refractive index and total solids shown in figure 1 is due chiefly to errors in determinations of solids, since any one set of determinations of solids, when plotted against refractive index, gave lines parallel to those of other sets. This suggests small variations in the drying treatment. A variation in the mineral constituents of the egg white solids may also account for part of the disagreement.

Romanoff (1929) has reported distinct differences in dry matter between the thick and thin white from the same egg. His results, which are based on the examination of only 5 eggs, most certainly do not agree with the results of our examinations. These, including a far greater number of eggs, show no exception, outside the limits of error, to the statements made above. The additional fact of equality in refractive index is conclusive support for the findings from determination of solids.

SUMMARY

The percentage of solids is the same in thick and thin white from the same egg, whether the egg is old or fresh. This conclusion is supported by the fact that the refractive indices are also the same.

The solids variation in fresh eggs was found generally in the range 10.7 to 12.9 per cent with extremes as low as 9.6 and as high as 13.5 per cent.

Refractive index measurements serve as a rapid means of estimating solids in egg white.

A rapid equilibrium with respect to water exists between thick and thin white in the same egg. The concentration of water remains the same in each regardless of losses to the yolk and through the shell.

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MEASUREMENT OF DETERIORATION IN THE STORED HEN'S EGG[1]

W. F. HOLST[2] AND H. J. ALMQUIST[3]

INTRODUCTION

If the meaning of the term 'freshness,' as applied to an egg, be restricted to indicate the degree to which the egg has retained its original internal and external quality during storage, then it follows that age, as a criterion of egg freshness, is excluded from consideration. This, within limits, is entirely justifiable, since variations exist not only in respect to the intrinsic keeping powers and initial quality of the individual egg, but also in the storage conditions to which the eggs may have been subjected. By the proper selection of eggs and a suitable control of their storage conditions, time as a factor governing the freshness of eggs may, for practical purposes, be to a certain extent eliminated. The other extreme can also be attained: eggs may be caused to deteriorate at a rapid rate. Unfortunately it is not usually feasible or profitable to modify commercial storage methods so as to achieve the optimum conditions. As a consequence certain undesirable processes may occur.

Several of the changes in the stored hen's egg are sufficiently marked to be noticeable to anyone concerned with the keeping of eggs at or near their original fresh condition. These changes are shrinkage, liquefaction of the thick white, and passage of water into the yolk.

Loss of water from the egg can occur without noticeable change in other respects. Furthermore, loss of water can be prevented, yet thick white liquefaction may proceed at a rapid rate. The loss of water or of carbon dioxide from the egg may be caused to take place, each in the absence of the other, by properly controlling conditions; hence it is possible to test the above statements experimentally. This

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was done by storing 30 eggs in a desiccator over calcium chloride in an atmosphere maintained at 5 per cent carbon dioxide. In this lot of eggs water was removed at a rapid rate by the calcium chloride, while the carbon dioxide concentration was kept constant. A similar lot of eggs was stored in a desiccator over 5 per cent sodium hydroxide solution. Under these latter conditions a high humidity was maintained while carbon dioxide was rapidly removed from the storage atmosphere and, in turn, from the eggs. After 26 days all eggs of the first lot had air spaces ¼ to ½ inch in depth, showing extensive shrinkage, yet the interior quality was excellent. Thick white constituted on the average 52 per cent of the total white. In the second lot very little shrinkage was apparent on candling; nevertheless the eggs were as a whole badly liquefied. The whites averaged only 30 per cent as thick white.

It was found experimentally that the eggs on which figure 4 is based showed rates of shrinkage not at all comparable to the keeping qualities as found in the yolk and in the thick white. A comparison of these lots of eggs is given in table 1. This is further evidence in favor of the view that loss of water is a relatively minor type of deterioration and is not, in itself, responsible for other changes which may take place in stored eggs.

TABLE 1

COMPARISON OF KEEPING QUALITIES WITH RATES OF SHRINKAGE OF EGGS STORED AT 86° FAHRENHEIT.*

Hen	General keeping qualities	Average per cent weight lost per egg per day
A	Excellent	0.678
B	Fair	0 627
C	Poor	0 542

* These are the same eggs as those shown in figure 4.

Holst and Almquist (1931) showed that the loss of water from egg white is uniformly distributed throughout thick and thin white, since the concentration of solid matter in one remains equal to that in the other with varying age of the egg, although increasing regularly with the disappearance of water. These changes in the whites are thus proportionate. *Hence, if shrinkage is the only change, the percentage of total white in the form of thick white will remain constant for any one egg.*

For the above reasons, shrinkage, the only one of the various storage changes which may be reliably detected by candling, is of little value as an index to egg quality, inasmuch as it often fails to parallel other departures of the egg from a fresh condition and becomes significant only in extreme stages.

It is well known that 'watery whites' are associated with weakened and easily broken yolk membranes, yet the extent to which these changes may be correlated with each other has, up to this time, not been demonstrated. The mechanism of thick white liquefaction is at the present time not well understood. However, certain relations between this liquefaction and yolk depreciation have been studied in order to establish a basis of comparison for methods which measure these types of deterioration.

The actual passage of water from the white into yolk may be shown by analysis. The average moisture content of the yolks of fresh eggs examined during some of this work was 48.02 per cent, as compared with a value of 54.33 per cent secured from the yolks of eggs stored for 10 days at 86°F. At the same time the average yolk weights increased from 15.62 to 17.58 grams.

The various changes in egg yolks are all attributable to osmotic forces operating so as to cause a passage of water from the white to the yolk. The water content of fresh yolk is in the neighborhood of 48 per cent, while that of fresh white normally is between 85 and 90 per cent. This difference in concentration of water creates a tendency for water to pass into and dilute the contents of the yolk membrane. One of the first to note this effect was Greenlee (1911). In the fresh egg, where the difference in water content is at the maximum, the osmotic forces are greatest but may largely be controlled by at least one other factor.

The water which thus diffuses into the yolk produces two effects, both of which are undesirable. To make room for the incoming water, the yolk membrane is compelled to stretch and is thereby weakened. Only in very rare cases, however, will this effect result in the breaking of the yolk while inside the shell. A second and perhaps more serious effect is the marked increase in fluidity of the yolk substance.

The fresh yolk, when the egg is broken onto a flat surface, will stand up well, but a yolk which has absorbed much water will slump down rapidly because of its increased fluidity. This slumping down causes the yolk to assume a flat shape much different from that of a sphere which, of all bodies, requires the least surface for a given

volume. A greater membrane area is thus suddenly required; this, in conjunction with the previously mentioned weakening of the membrane, often sets up a stress which the membrane cannot resist and the yolk breaks.

A further effect aiding those mentioned is due to the disappearance of all but small amounts of thick white as such in these serious stages of deterioration. The mechanical support offered to the yolk by firm thick white is lost as the thick white disappears.

METHODS OF MEASURING DETERIORATION

There remain then two trends which may be followed with assurance, i. e., the changes in the yolk and the changes in the thick white.

A method of measuring the first of these has been described by Sharp and Powell (1930). A factor which they call the yolk index, representing the quotient of the yolk height and yolk width as measured when the yolk is placed on a flat surface, apparently decreases with the progressive deterioration of the egg. It also decreases more rapidly with increasingly unfavorable storage conditions such as high temperature, etc. The lowering of the yolk index is probably directly associated with the passage of water into the yolk.

This procedure is confined to the yolk and furnishes no clew as to the initial condition of the egg in respect to the amount of thick white. To quote Sharp and Powell, "Some fresh eggs, however, may have a low interior quality, especially a watery condition of the white, so the standards of comparison must be modified to exclude such eggs."

This watery condition of the white in fresh eggs is of as much interest to the investigator as is the gradual liquefaction of eggs during storage. Our researches, though as yet of a preliminary nature as far as these properties, which are difficult to trace are concerned, lead to the suggestion that in the fresh egg the percentage of the total white which is in the form of thick white is directly related to the keeping qualities of an egg. Where other factors may be considered equal, the higher percentage of thick white indicates superior keeping qualities.

A group of 150 fresh eggs gave 62 as the average percentage of the total white existing in the form of thick white, while the individual values ranged from 45 to 90 per cent. Thus even fresh eggs vary greatly in this respect and, on the average, may have whites already 40 per cent liquefied.

If the same yolk is allowed to stand on a flat surface, the yolk index decreases continuously with time so that measurements must be made

after an arbitrary time interval. Sharp and Powell secured good agreement by working in this fashion. This feature, however, detracts seriously from the applicability of such a test to experimental work.

Furthermore, the yolk must be carefully separated from the white and dried with a towel while held in the hand. The assumption that this excessive handling has no effect on the yolk is questionable. Our experience has shown that yolks far removed from a condition of freshness can be so handled only with great risk of breaking them. It is much easier to remove the yolk cleanly from the surrounding white and measure the white itself.

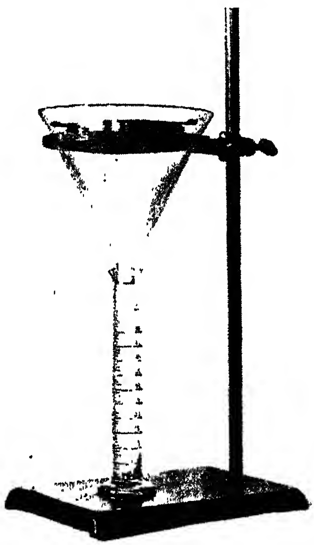

Fig. 1. Assembled apparatus used for the separation of thick and thin white and their volumetric measurement.

A second method of following storage depreciation, one which also furnishes an idea of fresh quality, consists of measuring the amount of white present in the firm jelly-like condition known as thick white. This procedure has been used in this laboratory for some time.

The apparatus required is shown in figure 1. It consists of three simple parts: a funnel, a graduated cylinder, and a specially constructed sieve. The only important specifications are those of the sieve (fig. 2), which has a diameter of 4 inches, a ½-inch raised rim, and a mesh of 9 per inch. The three tabs on the rim are for supporting the sieve inside of the funnel.

For the purpose of making measurements of thick white the egg is first broken into a standard-sized Petri dish. The yolk is removed, care being taken to separate the yolk and leave in the dish any adhering white. The white is then poured into the sieve mounted in the funnel which in turn delivers into the graduated cylinder. Thick white in good condition does not penetrate the sieve. In practice the sieve was gently rocked by hand in order to insure that thin white, which has the same density as thick white, was not held away from the openings by thick white. In a few seconds all thin white runs through.

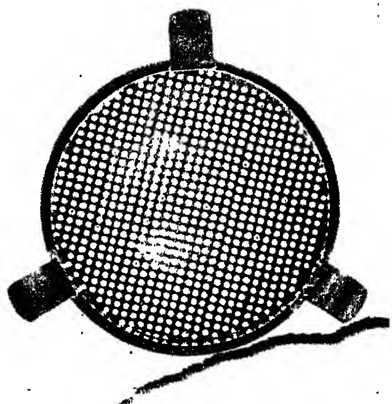

Fig. 2. Sieve used in the separation of thick and thin white in the apparatus shown in figure 1. The sieve itself is one used by Dr. S. L. Parker in her studies on individual and seasonal variations in the thick egg white (unpublished data).

When the penetration by thin white has slowed to a drop every few seconds the volume in the graduated cylinder is read to ½ cc. The sieve is then tilted and the thick white also allowed to run into the cylinder. The difference between first and final volumes represents the volume of thick white. This, expressed as percentage of total volume, gives the figures used at this laboratory to express the interior quality of eggs. Since, as previously mentioned, the densities and the concentration of solid matter are the same in both types of white from any one egg, the volume percentage of thick white is the same as the weight percentage. It is, furthermore, independent of shrinkage.

One source of error is the white unavoidably left behind in the petri dishes because of incomplete drainage. This error is small and assumes constancy for all eggs when dishes of the same size are used. A second source of error is due to the white required to wet the sieve and the portions of the funnel and cylinder with which the

white comes in contact, but this error may be balanced out by running the egg whites through as rapidly as proper measurements can be taken, keeping the apparatus wet. Thus the error due to this effect will apply only to the first and possibly the second measurements.

A fair degree of reproducibility may be obtained by this method in evaluating the condition of eggs which may be expected to be closely similar and in getting the same results by repeated trials with the same egg. In table 2 are summarized the results from measurements on series of eggs from the same birds. These eggs were all about one day in age.

TABLE 2

THE DISTRIBUTION OF THICK WHITE IN FRESH EGGS

Hen No.	Volume in cubic centimeters		Per cent thick white	Hen No.	Volume in cubic centimeters		Per cent thick white
	Thick white	Total white			Thick white	Total white	
C-52	21.0	36.0	58	C-21	15.5	25.5	61
	18.0	31.0	58		15.0	27.0	56
	19.0	33.0	58		15.5	25.5	61
D-965	22.5	36.5	62		15.5	26.0	60
	27.0	42.0	64	A-60	19.5	31.5	62
	23.0	36.0	64		18.0	29.5	61
	20.5	33.0	62		17.0	27.0	63
1370	18.0	22.0	82		17.5	28.0	62
	14.0	18.0	78	B-77	16.0	27.0	59
	15.0	20.0	75		14.0	24.0	58
	17.5	21.5	81		16.0	26.5	61
B-125	24.0	37.0	65	D-842	12.0	25.5	47
	21.0	33.5	63		12.0	25.0	48
	22.5	35.0	64		13.0	27.0	48
	24.0	39.5	62		11.5	23.5	49

It is worthy of note that the percentage column of table 2 shows in each series the least variation as compared to the other two, showing that the thick white percentage tends to be independent of variations in total amount of white and in egg size. Obviously both the eggs and the method must be very uniform to achieve these results.

EXPERIMENTAL METHOD

Since it is well known that thick white disappearance and passage of water into the yolk are found to occur together, it became of interest to secure some conception, first, of the rate of these processes, and second, as to whether they are simultaneous or not.

To investigate these questions the method last described above was used to detect changes in the thick white. Following the simplest manner of detecting the passage of water to the yolk, the yolks were carefully removed from the egg, freed from adhering white, dried briefly by rolling on soft absorbent paper, and weighed. Yolks from eggs which had been stored for some time often broke, even with the most careful treatment, making it necessary to discard all data from the eggs which supplied these yolks.

The storage was carried out at two temperatures, 64° F and 86° F. The humidity was kept constant and the carbon dioxide was removed by a 15 per cent solution of sodium hydroxide kept in the storage space. These temperatures are, of course, much higher than commercial storage temperatures, but have the advantage of shortening the experimental period by accelerating processes which occur at much slower rates under conditions more favorable to the keeping of the egg.

At the lower temperature data were taken at approximate 5-day intervals over a total period of 25 days, while at the higher temperature data were taken at 2-day intervals over a 10-day period.

On the assumption that a single bird generally would produce an egg of uniform characteristics, the work was broken up into a series of studies of the eggs from individual birds. This was expected to reduce as far as possible, the influence of variables such as egg size, shell texture and porosity, initial percentage of thick white, yolk weight, and other probable, but as yet unknown, sources of deviation. Results which are much clearer cut may be obtained by working in this way; those which have been obtained are a justification of the assumption. They were distributed among the various storage periods in a uniform manner, so that any one set of measurements usually would give data on eggs from all the different times of storage.

None of the eggs were protected in any way such as by oil dipping.

The data secured have been condensed and presented in graphic form in figures 3 and 4. Each point on these curves represents the average condition of at least four eggs at the designated time. For all but a few of these points the number of eggs is five or more.

Not all of the data have been shown, since to do so would result only in a repetition of certain type cases. Enough has been included to represent the extreme and mean cases and the trends common to all.

DISCUSSION

Figures 3*A*, at 64°F, and 4*A*, at 86°F, demonstrate that where thick white liquefaction does not occur, the osmotic processes by which water enters the yolk are inhibited, as shown by the fact that the average yolk weight does not increase. Figures 3*B*, 3*C*, 4*B*, and 4*C* show that when these phases of deterioration do take place they proceed simultaneously and to a corresponding degree.

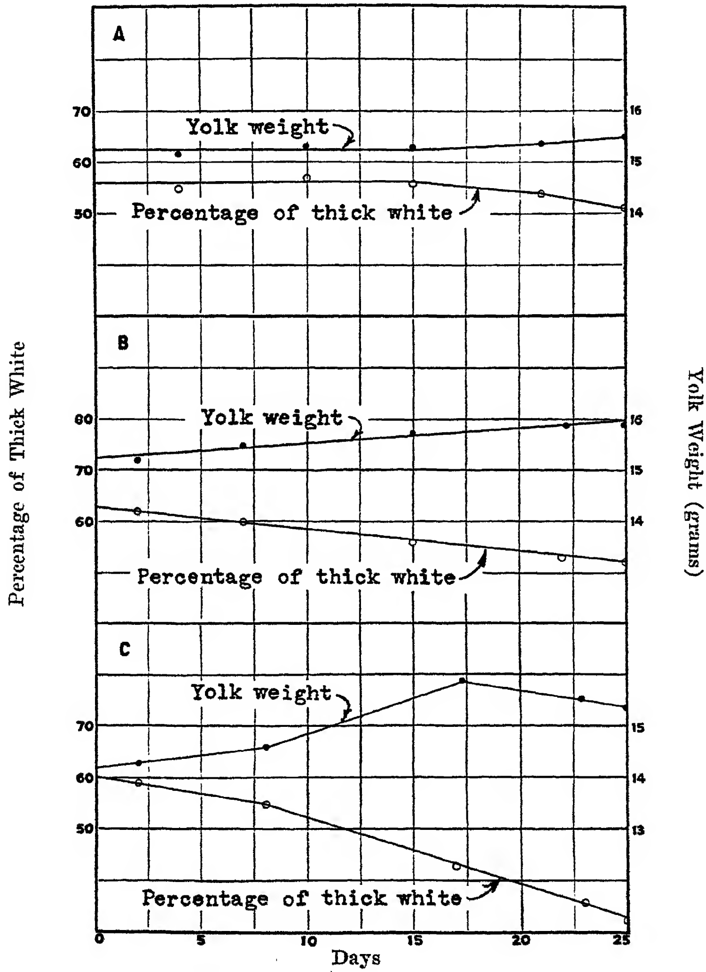

Fig. 3. The changes found in eggs stored at 64° F. Each graph represents data obtained from a series of eggs produced by the same hen. Circles represent percentage of thick white and the filled circles represent yolk weights. Each point shows the average condition of about 5 eggs at the designated time.

The apparent drop in yolk weight in the older eggs as shown in figure 3*C* is probably to be explained by the unusually high shell porosity of the eggs used in obtaining this graph. Under conditions of high porosity the entire egg system, yolk included, may be expected to lose weight after some time in storage.

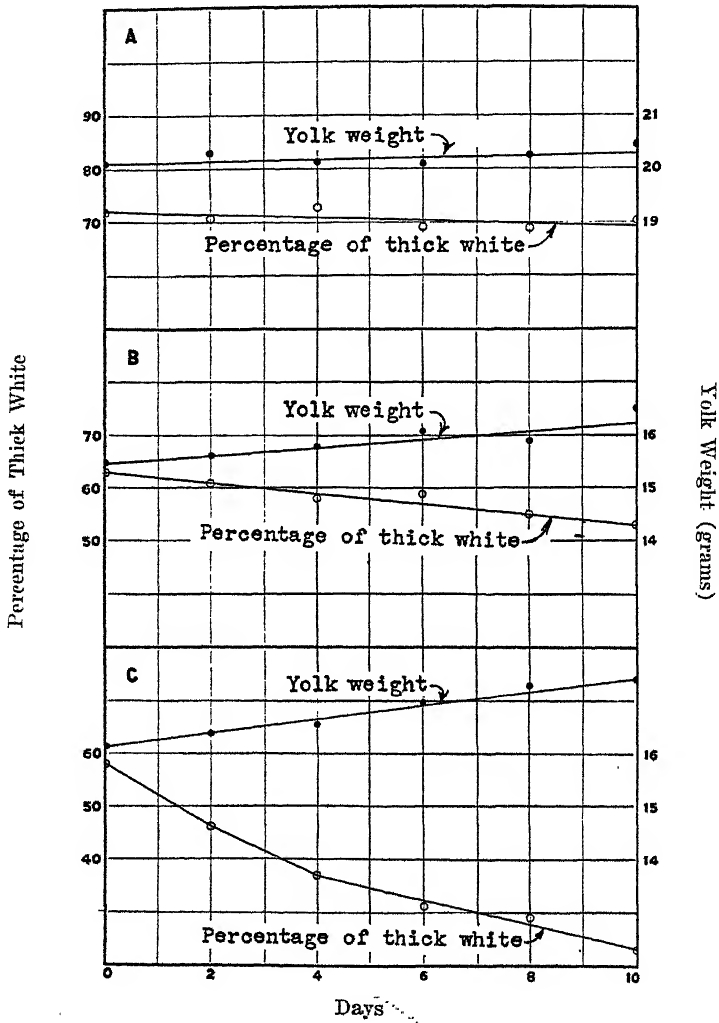

Fig. 4. The changes found in eggs stored at 86° F. As in figure 3, each graph represents the data obtained from a series of eggs produced by the same hen. Circles represent percentage of thick white and the filled circles represent yolk weights. Each point shows the average condition of about 5 eggs at the designated time.

Figures 3*A* and 4*A* are especially interesting in that, although these particular eggs were subjected to the same unfavorable conditions as the others and suffered shrinkage to comparable degrees, they have, nevertheless, demonstrated a high intrinsic keeping power.

The marked differences in the various egg series cannot be explained on the basis of variations in the feeding, housing, etc., of the hens from which the eggs were taken, since these factors were very uniform in these cases. The presented evidence points strongly toward the conclusion that the intrinsic keeping quality of an egg is to be added to the list of characteristics already known to be markedly influenced by the individuality of the hen.

It is apparent from the curves shown that the jelly-like structure of thick white begins to break down as the tendency of water to diffuse into the yolk becomes operative, therefore, *loss of quality in the yolk is accompanied by a corresponding loss in the white.* This is true for every case studied during this work.

The first explanation of these facts which occurs is that the water in thick white, due to the peculiar properties of this jelly-like substance, is held in such a manner that it cannot diffuse into the yolk as long as the thick white is well preserved. This, however, cannot be the true condition since it has been shown (Holst and Almquist, 1931), that the concentration of water in thick white remains exactly equal to that in the associated thin white regardless of losses to the yolk and to the atmosphere. Hence the activity of water in thick white is at all times equal to that of the water in the accompanying thin white, which in turn is nearly equal to that of pure water. The control of the tendencies which may bring about the diffusion of water to the yolk must lie within the yolk itself and may be connected with the increases in alkalinity known to occur in stored eggs.

A logical conclusion concerning the methods discussed is that the two expressions of storage deterioration in eggs, i. e., yolk index and thick white percentage, are correlated, but only as they follow changes which are contemporary. The superiority of the latter measurement lies in the following advantages:

(*a*) Better evidence regarding the initial fresh condition of eggs in respect to an important component, the thick white.
(*b*) Greater simplicity and speed in operation.
(*c*) Less danger of losing the measurements through breakage of the yolk.
(*d*) No necessity of making measurements in a specified time after the egg is opened.
(*e*) Independence of the measurement from shrinkage.

SUMMARY

Shrinkage has little significance as an index to egg quality.

Thick white percentage as an expression of egg quality possesses several points of superiority over the yolk index.

Liquefaction changes in thick white and yolk in stored eggs occur simultaneously or not at all.

The intrinsic keeping quality of an egg is markedly a function of the individuality of the hen.

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VARIABILITY OF SHELL POROSITY IN THE HEN'S EGG[1]

H. J. ALMQUIST[2] AND W. F. HOLST[3]

INTRODUCTION

In connection with the formation of the egg in the domestic hen, Surface (1912) state that the uterus, which is the shell-forming part of the oviduct, possesses at least two kinds of glands, the function of which is to furnish shell-forming material. From one kind calcerous matter is secreted, from the other mucus. The result is a shell which, according to Lillie (1919), consists of three layers, the mammillary layer, the intermediate spongy layer, and the surface cuticle. This heterogeneous envelope is permeable to gases. Lillie explains this characteristic on the basis of a supposed 'network' of pores in the spongy layer, connecting the conical inner ends of the mammillae with pores of the cuticle. This conception of shell porosity no doubt originated with Landois (1865).

The application of the term 'spongy' to the intermediate layer of the egg shell was due to an entirely faulty and misleading experimental procedure. Egg shells were treated with dilute mineral acids. As a matter of course small bubbles of gas, carbon dioxide, appeared scattered all over the exposed shell surface. These bubbles, however, were wrongly interpreted to indicate cavities in the shell and were further assumed to be interconnected by a network of fine channels. Thus, unfortunately, the name 'spongy layer' was introduced into ornithological terminology. Clevisch (1913), in the course of his much more thorough studies of the subject, found the intermediate layer to represent calcium carbonate crystals, densely knitted together by what appeared to be albuminous material. While observing no evidence of a porous network in the intermediate layer he did find

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it to be penetrated by small, definitely tube-like passageways. These passageways or pores were closed at the outer ends by the cuticle which appeared as a nonporous, structureless deposit or seal of a thin and delicate character. It could easily be assumed that a number of outside influences might partly or wholly remove this deposit, thereby exposing the pores.

Investigations by Rizzo (1899) showed that the number of channels in the shells of hen's eggs is very large, varying in the neighborhood of seven thousand per egg. His experimental method was such as to detect all pores, whether originally closed or not. Rizzo used a dye solution, with which he filled the empty egg shell. He then sealed and warmed it. The duration of these experiments, together with the elevated temperature and the pressure developed by expansion of the filling fluid on warming would be expected to cause penetration by the dye at all possible points. Thus the results of Rizzo would represent a uniform upper limit of porosity which might be termed the total or 'potential porosity.' It is apparent from his work, however, that at a somewhat smaller number of points the dye solution used penetrated without assistance to the exterior of the shell, indicating that some of the channels were partially or totally unobstructed at the time of examination. The actual porosity was, therefore, somewhat less than the total or potential porosity found.

On the reasonable supposition that the calcareous portion of the shell is traversed by a large number of these small channels, many of which may be obstructed by organic matter such as the cuticle, it appears that porosity may be a variable, *even in a given individual egg*, depending upon the natural characteristics and treatment of the egg. Thus, if it be recognized that porosity may increase, the limiting value approached may be expressed as the 'potential porosity,' that is, the condition found by Rizzo.

Storage of the egg in air may be expected to lead to drying and shrinking of the external seal, the cuticle, in this way partially opening the already existing and numerous channels. From this physical conception of porosity it will readily be seen that porosity variation of at least three types may result; namely, variation with time, variation with treatment, and variation which may be expected to exist between individual eggs.

In discussing egg-shell porosity, therefore, it is essential to give a definition of the term 'porosity,' although of necessity it will be an arbitrary definition. The term, as used in this publication, is meant to characterize the condition of the egg shell with regard to the num-

ber and distribution of those small channels or pores which, at the time of inspection, offer free passageway to water vapor, air, and carbon dioxide.

The present study, dealing with porosity conditions of hens' eggs investigated under what is believed to be a somewhat original viewpoint, should be of considerable fundamental importance. It is hoped that the information obtained may aid the study of egg quality. This is because porosity—or lack of porosity—must be assumed to be one of the important factors bearing on commercial egg quality and particularly on the preservation of eggs. Of course, other factors, in the egg itself or connected with the condition under which the egg is being stored, must also be assumed to be of significance.

METHODS OF MEASURING SHELL POROSITY

Several methods of measuring egg-shell porosity described in the existing literature were tried out but were later discarded. It is undesirable to apply to the shell any method involving an increase or decrease of pressure, because there is great likelihood of altering the existing porosity by dislodging the pore-filling material. For this reason the method which consists of placing the shell under water in a closed vessel and counting the bubble streams as pressure is reduced is open to objection. It is also unsuitable because of the difficulties in counting the bubble streams and the lack of a permanent record in the shell itself after the test has been made. The use of water or of aqueous solutions is in itself questionable since the effect of water in swelling the mucus in the pores, and later perhaps dissolving it, cannot be determined. Attempts were made to measure the rate of diffusion of gases through the empty shell. The results were inconsistent, largely owing, it was found, to the variation in the degree of dryness of the shell, a variation which can not easily be controlled.

Rizzo's use of a dye solution had great advantages, although his method of application was not satisfactory for the purposes of this work. The method described by Weston and Halnan (1927) of first painting the outside of the shell with a starch solution and later the inside of the shell with a solution of iodine represented an improvement over Rizzo's procedure. The method finally developed and adopted for routine observations in this laboratory, however, consisted of immersing the egg for 2 minutes in a solution of methylene blue in 95 per cent alcohol (3 grams per litre). After immersion the egg is allowed to dry, which requires but a short time. The shell

is then carefully split into halves and the contents are poured out. If the inside of the shell is wiped dry as soon as the egg contents have been removed, a clear and permanent picture of the porosity of the shell remains. An additional immersion of 3 minutes does not bring out more pores, but the spots where penetration has already taken place become enlarged because of diffusion of the dye in the shell membrane.

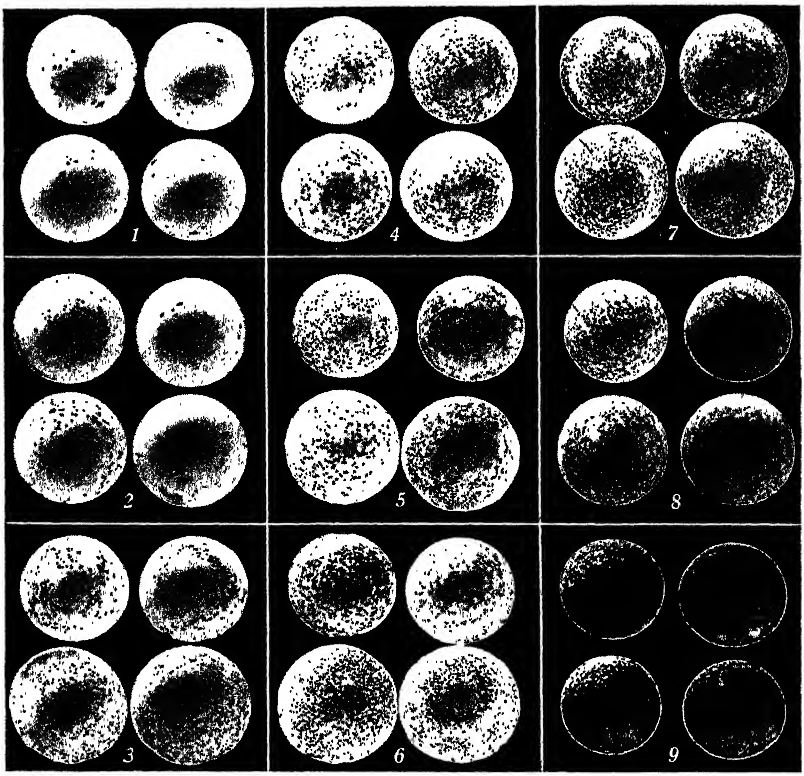

Fig. 1. Shell-porosity standards: the insides of shells adopted as standards of comparison. The numbers 1 to 9 represent increasing shell porosity as shown by the increasing number of small spots on the interior of the shells. Each small spot indicates an open pore. In each porosity class are included a large and a small shell, both halves of each shell being shown. Air-space ends are placed on the left.

In order to classify the shells examined for porosity in this manner a set of standards, covering the range of porosity encountered in this work, was selected and given arbitrary numbers. Later determinations of porosity were compared with these standards and assigned the corresponding numbers. The standards are reproduced in figure 1.

The main advantages of the method are speed, permanence of record, and availability of the egg contents for further inspection. As justification for this method of estimating porosity the following considerations may be advanced:

1. The protein materials which comprise the nonmineral substance of eggs are not soluble in alcohol of this concentration (95 per cent). The mechanism by which the dye penetrates the shell is therefore not one of dissolving the protein-like, pore-filling substance.

2. The alcohol might exert a dehydrating and coagulating action on the cuticle. This would produce an effect similar to ordinary drying and shrinking in reducing the effectiveness of this pore-sealing material. However, it does not seem that this type of action could be effective in the short period required for the test; furthermore, if such action were effective, then, in view of the fact that the potential porosity of all normal egg shells is a fairly uniform and high value, all eggs tested would be expected to show a much greater and more uniform response to the treatment than is actually found to be the case. The variations of porosity and the differences in distribution, as presented later in this article, would be either much less prominent or nonexistent.

3. Observations which have been made by partially submerging emptied half shells in the dye solution have shown that penetration begins at once and that the spots produced merely enlarge during the remainder of the test period.

4. Finally, the relation which the measured porosity has shown to the rate at which eggs lose weight seems to justify confidence in the idea that this method of estimating shell porosity is based upon a fundamental property of the shell, namely, the effectiveness of the shell channels as free pores.

The loss of weight in stored eggs is undoubtedly controlled by the shell porosity. To check the method just described the relation between weight loss and estimated porosity was examined in a number of eggs.

In table 1 are summarized data secured under the direction of the authors.

Eggs were stored 5 to 6 days at 86° F and at constant humidity. Loss of weight was determined by accurate weighings and expressed as percentage of fresh weight lost per egg per day. The porosity numbers were assigned as described previously in this article. The results of the two determinations were collected for some time in an independent manner, then brought together, calculated to a common basis, and compared.

TABLE 1

THE RELATION OF SHELL-POROSITY NUMBER TO THE MEASURED LOSS OF WEIGHT IN HEN'S EGGS

	Porosity numbers*								
	1	2	3	4	5	6	7	8	9
Total number of eggs of each porosity rating.	1	3	16	20	22	18	16	15	11
Average per cent of weight lost per egg per day .	0 212	0 442	0 502	0 532	0 573	0 603	0 691	0.917	1.047

* See figure 1 for shell-porosity standards.

The table clearly illustrates a parallel trend in assigned porosity numbers and measured loss of weight. If the loss of weight is plotted against porosity number (fig. 2), it will be seen that a practically linear relation exists over the range 2 to 7. The loss of weight increases sharply with the porosity numbers 7, 8, and 9, indicating that these notations represent greater increments in porosity than do the preceding ones. Perhaps this range should be subdivided into a somewhat greater number of comparative standards to achieve greater consistency with the lower porosity numbers.

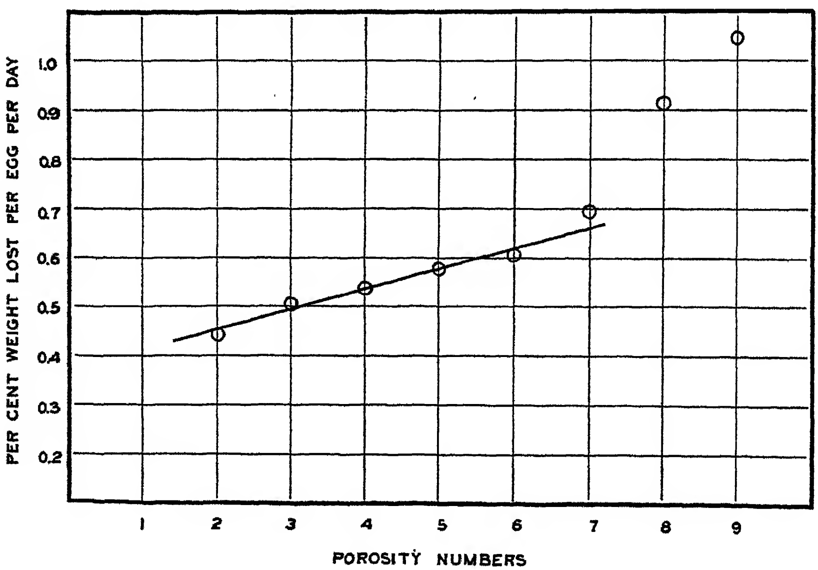

Fig. 2. The agreement between the assigned shell porosity numbers and the measured rate of losing weight in 122 hens' eggs at 86° F.

It cannot be claimed for this method of evaluating porosity that an absolute measure is achieved. Although tests may be conducted in a uniform manner the final ratings depend upon personal judgment. Human errors may be reduced by working with larger numbers of eggs and in a random manner so that preconceived ideas may not prevail in classifying a particular egg or the egg from a particular hen. As far as possible this was done. It is a matter of practical impossibility to make an actual count of pores in a large number of egg shells. The method of classification just described sacrifices accuracy for speed in the hope that, on the average and with large numbers of eggs, results may still be significant.

The procedure used gives an approximate quantitative measure of variability in shell porosity. It has also shown certain changes in shell porosity with time and temperature not previously reported.

RESULTS

Eggs from a group of more than 50 hens were examined. Included in this group were eggs showing wide differences with respect to shell characteristics, such as thickness and smoothness. In all more than 500 shells were examined for porosity. At the same time data were taken regarding shrinkage and keeping qualities. The latter work is still in progress.

Fresh Eggs.—The examination of fresh eggs demonstrated a striking variability in porosity in eggs from different birds. Eggs from the same bird were usually found to be fairly uniform in this respect.

Statements in the literature create the impression that shell porosity is greater or that pores are larger at the air space (Rizzo, 1899; Dunn, 1923; and Swenson and Mottern, 1930), but observations made in this laboratory on eggs one day or less in age indicated that this condition is far from being general. There is no apparent fundamental reason for the existence of greater porosity at air spaces in fresh eggs, but this may be expected in older eggs, for, at the air space, the shell is not in contact with watery material but is bounded on both sides by gases. This probably leads to a faster drying of the shell and of the pore-filling material and a corresponding faster opening up of the pores in this part of the egg.

Figure 3, *A* and *B*, represents shells in which very little porosity was found at the air space. The position of the air space is indicated by the circle. Both halves of the shell are shown, the air space ends being placed on the left in these and in all other cases.

Figure 3*C* illustrates some of the few cases in which porosity was found to be distinctly greater at the air-space end. It will be noted that the great degree of porosity is not necessarily confined to the shell portion forming the air space.

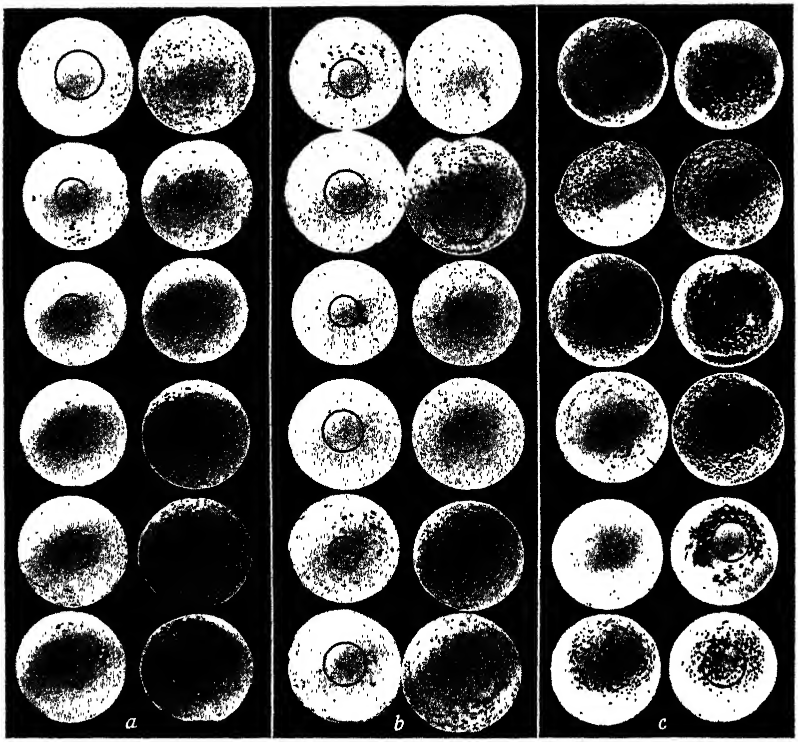

Fig. 3. Abnormal distributions of porosity in shells of fresh eggs. Both halves of each shell are shown. The air spaces occupied the regions indicated by the circles.

The standards (fig. 1) illustrate the type of porosity most commonly found in fresh shells. About 80 per cent of the shells examined showed a uniformly distributed porosity of this nature. The variability of this porosity in fresh eggs is indicated by the standards. All degrees of porosity represented by these figures were found in fresh shells, the condition most frequently met, however, being represented by porosity numbers 4 and 5.

Stored Eggs.—In order to note possible changes of shell porosity during storage, eggs were stored at different temperatures—at room temperature (about 68° F), at 86° F, and at 102° F. At the last two

temperatures the humidity was maintained at about 75 per cent. Examination of the egg shells after storage revealed certain significant facts.

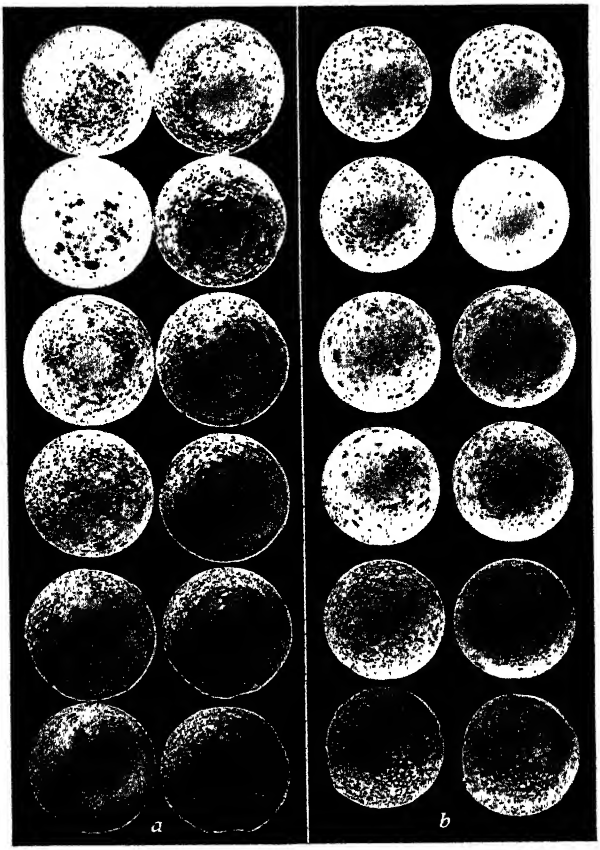

Fig. 4. Increases in porosity found at room conditions over a twenty-five day period. Egg age increases in five-day increments from top to bottom of figure. Air-space halves are placed on the left. All shells in *a* are from one hen, all shells in *b* from another.

While heretofore porosity has been considered a rather fixed characteristic of the shell, it was apparent that porosity is not necessarily constant, that it may increase with the age of the egg, and more rapidly with higher storage temperature. A few exceptions to these findings resulted when eggs from the same hen started at a rather high initial prorosity and maintained this at about the same level over a period of 25 days, when stored at room conditions.

The increases in porosity as observed may partly explain the abnormally poor keeping quality of eggs which have been removed from storage. In particular eggs stored under conditions of low humidity would probably show an increase in porosity which would greatly favor their deterioration upon removal from storage.

In figure 4 are shown two series of shells, each series from an individual bird, which illustrate porosity increases encountered in eggs stored at room conditions over a period of 25 days. The shells are 5 days apart in storage age.

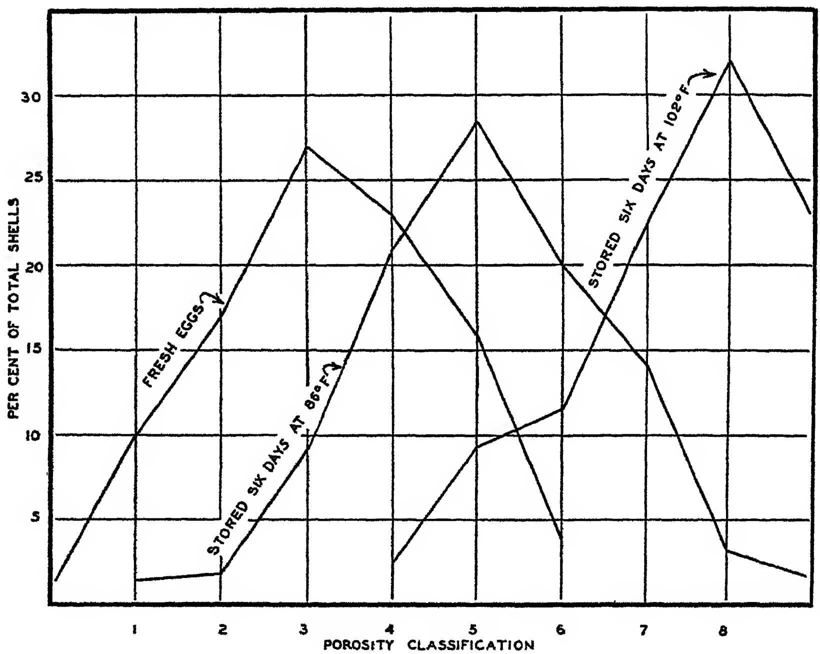

Fig. 5. Distribution of porosity in fresh eggs and eggs stored for six days at 86° F and at 102° F.

It is possible that the apparent increases in shell porosity with age may be due to differences in initial porosity. The changes noted, however, were found to be general, and for the most part the porosity in the older shells is far greater than the average initial porosity of eggs from the same hen. A more uniform distribution of the pores in the shell is invariably associated with an increase in degree of porosity.

In order to express the effects of storage on porosity at the various temperatures quantitatively, the results of an investigation have been shown graphically in figure 5. In this figure, one curve represents the

distribution of porosity in 72 fresh shells, one day or less in age, which came from a group of 12 hens used in this work. Six eggs were used from each bird. A second curve shows the distribution of porosity found in an equal total number of shells and with the same individual number from the same birds, after the eggs had been stored for 6 days at 86° F. The third curve shows results from inspection of random eggs, largely from the same hens, after 6 or 7 days at 102° F. The first and second curves are entirely comparable. The shift of the most probable porosity toward a higher value after 6 days' storage is significant of a tendency toward an increase in porosity. It is also noteworthy that the porosity of eggs at incubation temperature (102° F) tends to reach a still higher value in a similar period of time.

SUMMARY

A new method for the study of egg-shell porosity has been suggested.

The shell porosity in fresh eggs, i. e., the initial porosity, with but few exceptions, has been found to be low.

Egg shells are subject to changes in porosity when the eggs are stored. Shell porosity may increase with duration of storage, more rapidly at higher temperatures, and approach a maximum which is nearly uniform for all eggs with regard to degree and distribution.

Egg-shell porosity appears to be nearly uniform for the eggs of a particular hen, but shows differences for different individuals.

Porosity in fresh egg shells is rather uniformly distributed. It is not generally greater in the air-space region of the egg.

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FACTORS AFFECTING CALIFORNIA RAISIN SALES AND PRICES, 1922-1929[1]

S. W. SHEAR[2] AND R. M. HOWE[3]

At what price can the tonnage of California raisins available during any particular marketing season be sold? As important as this question obviously is to those producing and marketing California raisins, many of the basic data needed in its solution were unavailable until July, 1930. At that time, however, through the cooperation of the members of the Dried Fruit Association of California, the independent packers of the state, and the Sun-Maid Raisin Growers Association, records of the quantities of California raisins sold for the crop years 1921–1929 and of the actual f.o.b. prices received were made available to the Giannini Foundation. Together with other more readily available information these data have been used as the basis of the present attempt to discover and measure the influence of the factors that have determined the quantities of California raisins sold annually in the domestic and in the overseas markets during the last eight marketing seasons, 1922–1929.

Although the analysis explains only what has occurred in the past, much of its value obviously lies in the help it can give the industry in judging the price at which any given tonnage may be expected to sell during any given crop year in the future. In fact, the specific reason for undertaking the study in the spring of 1930 was to make available a better basis for such judgment in the proposed control program of the industry.

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[1] Paper No. 20, The Giannini Foundation of Agricultural Economics. This study was made with the financial cooperation of the Federal Farm Board.

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CALIFORNIA RAISIN SALES

Within a decade California raisin production has nearly doubled. As a consequence the industry has experienced drastic price declines. Production averaged about 285,000 tons during the years 1926, 1927, and 1928, or over 100,000 tons more than the average at the close of the War.

TABLE 1

COMPLETED SALES OF CALIFORNIA RAISINS BY COUNTRIES, 1921–1929*

Year beginning Sept. 1	Grand total	Domestic			Exports			
		Total, U. S. and Canada	United States	Canada	Total		United Kingdom	Other countries
					Including Canada	Excluding Canada		
	Nearest hundred short tons, sweat-box basis*							
	1	2	3	4	5	6	7	8
1921	155,000	139,700	125,200	14,500	29,800	15,300	11,000	4,300
1922	190,000	153,500	135,000	18,500	55,000	36,500	20,400	16,000
1923	195,000	168,400	149,400	19,000	45,600	26,600	8,300	18,300
1924	220,000	187,600	167,600	20,000	53,000	32,400	14,800	17,600
1925	240,000	185,300	168,000	17,300	72,000	54,700	23,800	30,900
1926	245,000	182,400	162,300	20,100	82,700	62,600	28,500	34,100
1927	285,000	199,600	178,000	21,600	107,000	85,400	37,700	47,700
1928	290,000	193,400	171,000	22,400	119,000	96,600	37,000	59,600
1929	215,000	162,800	148,500	14,300	66,500	52,200	19,100	33,100

* Sales have been converted to a sweat-box basis by multiplying the net weight of packed raisins as sold by 1.08 or the gross shipping weight as reported by the carriers by 0.933. A wooden box of 25 pounds net weight of Thompson Seedless raisins weighs approximately 29 pounds gross (see Calpak Annual. July, 1930. p. 17). Sales through by-products channels and to other packers are excluded from these data.

Sources of data:

Col. 1: Total sales are based largely upon records of shipments of raisins from California by rail and intercoastal water as reported by carriers, and by direct export to foreign countries from the San Francisco and the Los Angeles customs districts, plus estimated California consumption based on per capita consumption in the rest of the United States. Reported shipments, however, have been checked against completed sales compiled by the Giannini Foundation from records of the Sun-Maid Raisin Growers Association and summarized sales of other raisin packers furnished by the Dried Fruit Association of California through the cooperation of its members.

Col. 2: Sum of items for corresponding years in cols. 3 and 4.

Col. 3: Items in col. 1 minus the items for corresponding years in col. 5.

Cols. 4, 5, 6, 7, 8: Compiled from U. S. Monthly Summary of Foreign Commerce. Net weight converted to approximate sweat-box basis by multiplying by 1.08.

In spite of the great decline in prices and the diversion of a considerable tonnage into by-products (alcohol, syrup and stock feed), the September 1 raisin carryover in the state has been in the neighborhood of 100,000 tons for the last four years (see table 3). Prices have not been low enough since 1920 to move all of the available supply for any crop year into consumption.

TABLE 2

PERCENTAGE OF CALIFORNIA RAISIN PRODUCTION BY VARIETIES, 1921–1930

Crop year	Total	Thompson Seedless	Muscat	Sultana	Others*
1921	100.0	49.1	38.8	8.9	3.2
1922	100 0	55.6	34 8	7.4	2.2
1923	100.0	60.7	30 6	7.5	1.2
1924	100.0	64.8	27.4	7.0	0.8
1925	100.0	77.0	14.9	6.4	1.7
1926	100.0	69.3	22.8	5.8	2.1
1927	100.0	71.4	22.0	5.2	1.4
1928	100.0	79.5	13.0	5.3	2.2
1929	100.0	73.7	20.3	4.3	1 7
1930	100.0	73.7	21.4	4.0	0 9

* "Others" may include some soda and oil-dipped Sultanas and Thompson Seedless.

Sources of data:

Computed from the total of Sun-Maid and packer receipts by variety as reported to the Giannini Foundation except 1930 data, which are based on receipts of the California Raisin Pool to February 28, 1931.

TABLE 3

UNSHIPPED STOCKS OF CALIFORNIA RAISINS IN THE HANDS OF SUN-MAID AND INDEPENDENT PACKERS ON SEPTEMBER 1, SOLD AND UNSOLD, SHORT TONS, SWEAT-BOX BASIS, 1921–1930*

Year	Total	Thompson Seedless	Muscat	Other varieties
1921	36,000	5,200	22,600	8,200
1922	34,000	9,400	19,500	5,100
1923	86,000	40,900	40,300	4,800
1924	186,000	107,300	64,500	16,200
1925	67,000	37,400	20,600	9,000
1926	59,000	48,500	3,600	6,900
1927	108,000	81,400	15,400	11,200
1928	124,000	91,700	28,700	3,600
1929	92,000	73,800	11,000	7,200
1930*	92,000*	67,000*	18,500*	6,500*

* An actual inventory of 117,000 tons of raisins on May 31, 1930 was reported, of which about 85,300 tons (73 per cent) were Thompson Seedless, 23,000 tons (20 per cent) Muscats and 8,700 tons (7 per cent) other varieties, largely Sultanas. The inventory total as given for September 1, 1930, was calculated as explained below, and the variety totals by applying the May 31 variety percentage distribution to this total.

The carryover from the 1928 crop on September 1, 1929 was 92,000 tons. Mimeographed release No. 1245, June 7, 1930 of the Dried Fruit Association of California, shows actual receipts of 1929 crop raisins from growers by Sun-Maid and the independent packers up to about the last of April, of 215,000 tons. Completed and shipped sales from September 1, 1929 to August 31, 1930, were 215,000 tons, the same as receipts. Hence unshipped stocks in the hands of the packing industry on September 1, 1930, appear to have been at least 92,000 tons. They may have been slightly larger, since packers estimate that growers held between 5,000 and 10,000 tons of unsold raisins at the time the packing industry reported receipts of 215,000 tons this spring.

Sources of data:

Compiled from records of the Sun-Maid Raisin Growers Association and summarized data of other raisin packers furnished by the Dried Fruit Association through the cooperation of its members. Ninety-five per cent or more of the stocks of California raisins are accounted for by this table.

Thompson and Muscat Supply and Price Changes.—In the absence of adequate data on annual sales by variety, the percentage of receipts by varieties, as shown in table 2, gives the best available clue to changes in their relative importance. However, in order to visualize changes in the quantity sold by varieties, the carryover data by varieties, shown in table 3, must also be considered, as well as the fact that a majority of the by-products made from the 1923 crop surplus utilized Thompson Seedless. The rapid increase in the proportion of Thompson Seedless raisins from 49 per cent in 1921 to 74 per cent in 1929 and the corresponding decline in Muscat production from 39 to 20 per cent of total dried output of the state, helps to explain the fact that since 1925 the f.o.b. price of Muscats, as shown in table 4, has been higher than for Thompson Seedless. For at least fifteen years previous to 1925 prices of Thompson Seedless raisins and returns per acre were usually substantially higher than for Muscats. The greater returns from Thompson Seedless raisins during that period largely account for the tremendous increase in the production of this variety in California during the last twenty years, finally resulting in recent years in a somewhat adverse price differential as compared with Muscat prices.

PRICE CHANGES

Figure 1 shows not only the big increase in California raisin sales since the War but also the great decline in f.o.b. prices. The extreme decline from 14.0 cents in 1921 to 7.3 cents in 1923 reflects the artificially high raisin prices of 1921, the moderately adverse business conditions of 1923, the tremendous state crop of that year, and low prices in foreign countries.

Expansion of the total tonnage sold between 1923 and 1926, while average prices remained practically on the level, reflects increased export sales which were stimulated by the increasing differential by which California raisins undercut raisins from other countries in the chief export markets. (See fig. 4 and p. 88). Increased foreign demand, resulting largerly from Sun-Maid Raisin Growers Association's foreign sales campaign, has also helped to expand export tonnage since 1923. To maintain the average level of prices from 1923 to 1926, however, required considerable by-product utilization, largely from the bumper crop of 1923, and resulted in undesirably large carryovers (see table 3).

In spite of the bumper crop of 1926, California prices were maintained and sales, as a result, expanded but slightly. With a very large carryover at the end of the season and another bumper crop in 1927, prices were reduced to a 5.9 cent average for the season. The price cut, however, was not drastic enough to sell the available raisin tonnage, and, when the large 1928 crop was dried, a huge tonnage of the 1927 harvest was still on hand. When these facts and their possible effect on the Sun-Maid Raisin Growers Association became generally known, California raisin prices declined to a very low level, averaging about 4.7 cents for the season of 1928 as a whole. The low prices stimulated the sale of the largest tonnage of California raisins ever sold in a single year.

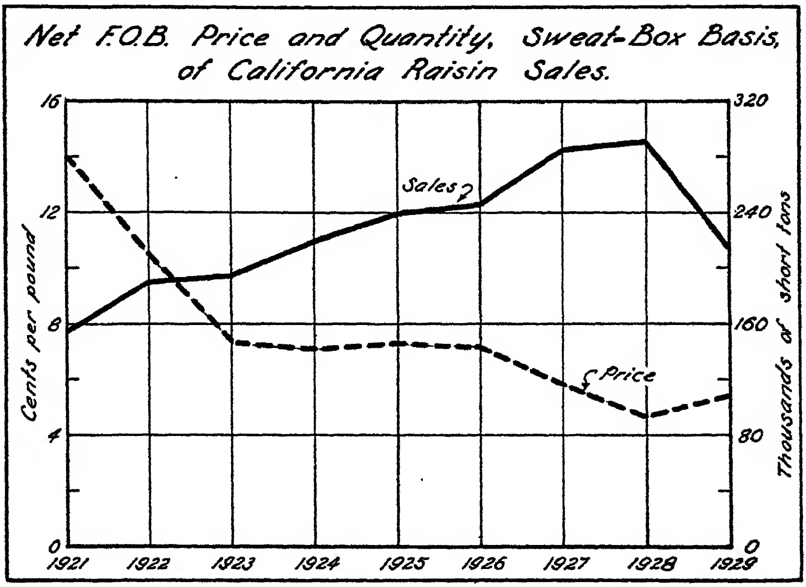

Fig. 1. Data for years beginning September 1, from tables 1 and 4.

In spite of low prices during the 1928 marketing season, the unsold tonnage was so large when the small 1929 crop was harvested that available supplies were even greater than the large tonnage sold in the 1928 marketing year. However, in the face of these supplies, the generally depressed business conditions both at home and abroad, and the large foreign crop which brought about drastic reductions in competitor's prices in the United Kingdom, particularly in Australian raisin prices, the price of California raisins unfortunately was raised in the summer of 1929. Apparently the California industry

underestimated foreign competition, the size of the carryover from the 1928 crop, and the unfavorable demand situation and possibly overestimated the probable influence of stabilization activities[4] for, although the average price of 5.4 cents for the 1929 season was relatively low, the tonnage sold was unusually small. Only about 215,000 tons were disposed of, or practically the equivalent of the 1929 crop, still leaving an inventory of about 92,000 tons of old raisins on hand in the state on September 1, 1930, to handicap the 1930 marketing

RELATION OF DOMESTIC SALES TO PRICES

Normally price is one of the most important factors determining the quantity of raisins consumed in the domestic market[6]. Figure 1 has already shown that prices have been low when the tonnage sold was large. The scatter diagram, figure 2, gives a more direct picture of the fact that high prices are associated with small consumption and low prices with large consumption. The quantity of raisins imported into the United States since September, 1922, and the quantity of raisins imported into Canada other than those originating in California, has been so small that it has been disregarded in this analysis. The free-hand curve *dd′* indicates the approximate relation between the quantities sold in the domestic market in the years 1922, 1924, 1925, 1926, and 1927, in which demand conditions affecting California's raisin markets were more favorable than in 1923, 1928, and 1929 and probably more favorable than they can be expected to average for several years, considering the prospects of low general price levels and also of large raisin crops and hence low prices for raisins from Australia and other foreign countries. This curve indicates that the domestic demand for raisins is inelastic, the elasticity at different points varying from approximately 0.3 to 0.4. It takes a relatively drastic cut in price, therefore, to induce any substantial increase in the amount consumed and large supplies return a smaller income to the industry than small supplies. Large crops of raisins are, therefore, extremely

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[4] See accompanying paper regarding certain of the activities of the Federal Farm Board and the California Grape Stabilization Board in 1929: Mallory, L.D., S. R. Smith and S. W. Shear. Factors affecting annual prices of California fresh grapes, 1921–1929. Hilgardia, 6:127. 1931.

[5] See table 3 and footnote for details regarding the carryover situation on September 1, 1930.

[6] Throughout this paper the United States and Canada together are considered as the *domestic* market in keeping with the usual practice of the California dried-fruit trade.

serious, since prices must be set very low in order to move them into consumption and growers receive very much less for large crops than for small ones.

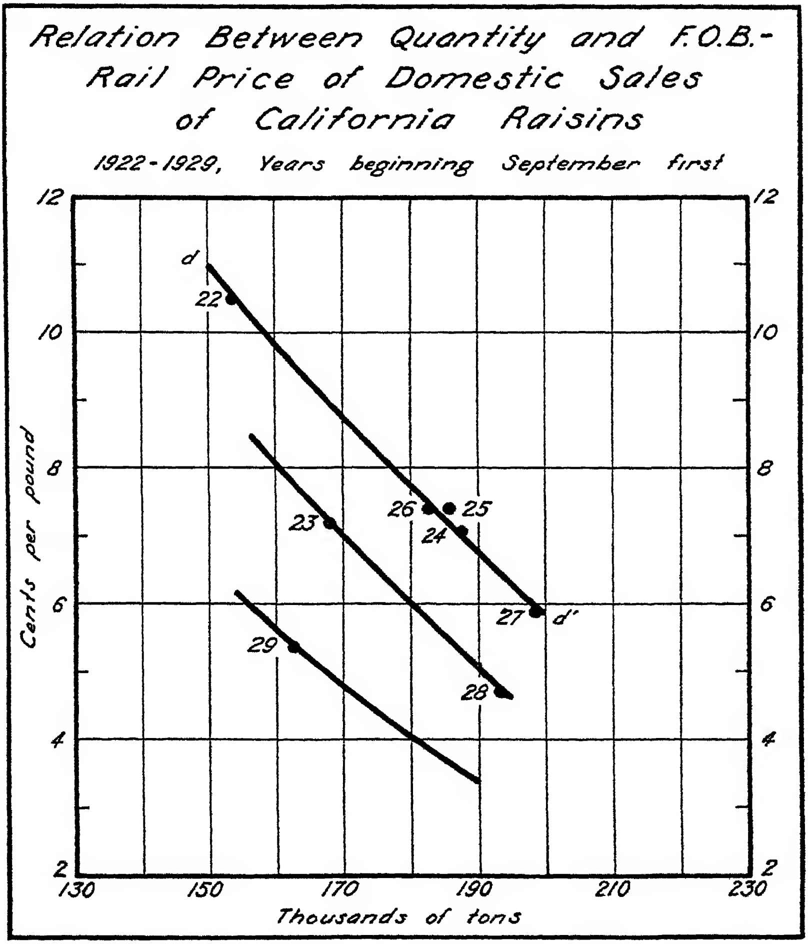

Fig. 2. Domestic sales include California exports to Canada. Data from tables 1 and 4.

The free-hand curve just below *dd′* passing through the 1923 and 1928 points approximates a demand schedule under demand conditions less favorable for raisin prices than the average for the years which *dd′* reflects. Generally adverse business conditions prevailing during the 1923 marketing season apparently account to a considerable

extent for the lower level of raisin prices in that year. In 1928, however, trade uncertainty was perhaps the most important depressive factor.

TABLE 4

CALIFORNIA F.O.B.-RAIL RAISIN PRICES, IN CENTS PER NET PACKED POUND, 1921–1929

Year beginning Sept. 1	Domestic and foreign sales: Grand total	Domestic and foreign sales: Thompson Seedless (natural)	Domestic and foreign sales: Muscats	All varieties: Domestic	All varieties: Foreign
	1	*2*	*3*	*4*	*5*
	cents	*cents*	*cents*	*cents*	*cents*
1921	14.0	*	*	14 0	13.5
1922	10.5	*	*	10.5	10.0
1923	7.3	*	*	7.2	8.0
1924	7.1	7.4	6.8	7.1	7.1
1925	7.3	7 3	7.7	7.4	7.2
1926	7.2	6.8	7.5	7.4	6.9
1927	5.9	5.6	6.7	5.9	6 0
1928	4.7	4.4	5.0	4.7	4.6
1929	5.4	4.9	6.3	5.4	5.4

* Data prior to 1924 were too incomplete to compute average prices for individual varieties but are sufficient to indicate that Thompson Seedless prices were higher than Muscat prices in the years 1921–1923.

Sources of data:

Compiled from the data reported on completed sales to the trade of Sun-Maid Raisin Growers Association and other packers by dividing money received (net, excluding cash discounts and brokerage) f.o.b. California rail shipping points by the corresponding tonnage of completed sales as reported on a net-weight basis. Sales through by-products' channels and to other packers are excluded from these averages.

Col. 1: Average of all varieties, types, grades and packs, including bleached, soda, and oil-dipped Thompson Seedless and Sultana.

Col. 2: Average of all grades and packs of natural Thompson Seedless, excludes bleached, soda, and oil-dipped.

Col. 3: Average of all grades and packs of Muscats.

Col. 4: Average of all varieties, types, grades, and packs sold in the United States and Canada.

Col. 5: Average of all varieties, types, grades, and packs sold in foreign markets, excluding Canada.

The lowest curve, passing through the 1929 point, indicates approximately the tonnage one might expect to sell in years in which general business conditions were as adverse as in 1929. Tonnage sales indicated by this curve, and the middle curve at half-cent price intervals, are shown in table 10. A similar schedule of the relation of domestic sales to f.o.b. prices can easily be constructed from the upper curves.

Obviously the determination of the shape of the free-hand curves in figure 2 involves a very large measure of individual judgment, since the points upon which they are based are very limited in num-

ber. This is particularly true of the two lower curves, which were included because of the probability that the level of demand may be expected to be more nearly at these lower levels during the next few years than near the higher level of *dd'*. The great decline in the general level of all commodity prices, beginning in 1929, is obviously in line with this reasoning.

EXPORTS TO FOREIGN MARKETS

Much of the increase in California raisin production and shipments since the War has been absorbed by overseas exports, that is, United States exports to all countries other than Canada[7]. Table 1 shows that the proportion exported to overseas markets rose from about 10 per cent in 1921 to over 33 per cent in 1928, the peak year of post-war exports. Only about 15,000 tons (sweat-box basis) moved to foreign countries in 1921, compared with over 96,000 tons in 1928.

United Kingdom, the Chief Foreign Market for California.—In recent years the United Kingdom has been the largest market for California export raisins, absorbing over 40 per cent of the total California overseas exports. During the last three years this one market has imported an average of nearly 31,000 short tons (equivalent sweat-box basis) of California raisins, or nearly one-eighth of the state's total sales tonnage and over one-third of the total raisin imports of the United Kingdom (see table 6). Because of its importance and representativeness, special study of this foreign market has been made in an endeavor to explain what determines the price of California raisins in European markets.

Until about 1924 Turkey[8] was the chief source of United Kingdom raisin imports. Australian production previous to that time was small and largely consumed at home. Therefore, it affected the world market but slightly. Since then, however, California and Australia have become the two most important sources, Turkey declining to about half its former importance. The large and increasing proportion of United Kingdom raisin imports supplied by Australia is shown in table 7. During each of the last two years they have amounted to nearly 47 per cent of the total.

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[7] As indicated in footnote 6, page 78, exports to Canada are included in domestic sales.

[8] A large part of Turkish raisins are exported from Smyrna and hence the trade frequently uses the term "Smyrna" raisins as synonymous with "Turkish" raisins.

TABLE 5

WORLD PRODUCTION OF RAISINS BY COUNTRIES, 1921–1930

Year harvested	Total	California	Total, foreign countries	Turkey (Smyrna)	Australia	Spain	Greece and Crete
	Production in short tons, dry weight						
	1	2	3	4	5	6	7
1921	219,900	145,000	74,900	37,400	9,400	12,100	16,000*
1922	324,600	237,000	87,600	41,200	15,100	15,300	16,000*
1923	383,500	290,000	93,500	44,300	20,900	17,300	11,000
1924	306,400	170,000	136,400	57,100	33,100	28,200	18,000*
1925	313,200	200,000	113,200	32,500	28,600	33,600	18,500
1926	391,700	285,000	106,700	39,200	25,100	25,900	16,500
1927	455,400	300,000	155,400	56,000	49,000	25,800	24,600
1928	395,700	268,000	127,700	49,300	27,600	25,200	25,600
1929	372,700	215,000	157,700	56,000†	59,000	20,700	22,000
1930‡	332,200	192,000	140,200	41,500†	59,000	17,700	22,000*
	Per cent of total production						
1921	100.0	65.9	34 1	17.0	4.3	5.5	7.3
1922	100.0	73.0	27.0	12.7	4.7	4.7	4.9
1923	100.0	75.6	24.4	11.6	5 4	4.5	2.9
1924	100.0	55.5	44.5	18.6	10.8	9 2	5.9
1925	100.0	63.9	36.1	10.4	9.1	10 7	5.9
1926	100.0	72.8	27.2	10.0	6.4	6.6	4.2
1927	100 0	65.9	34.1	12 3	10.7	5.7	5.4
1928	100.0	67.7	32.3	12 4	7.0	6.4	6.5
1929	100.0	57.7	42.3	15.0	15.8	5.6	5.9
1930‡	100.0	57.8	42.2	12.5	17.8	5.3	6.6

* Rough estimates based on crop-year exports of raisins from Greece and Crete and production condition of Greek currants for 1921, 1922, and 1924.

† It is estimated that 700 tons will be used by the Alcohol Monopoly from the 1930 crop of Turkish raisins compared with 13,900 tons of the 1929 crop. A portion of some previous crops have also been utilized for alcohol.

‡ All 1930 data are preliminary and subject to revision.

Sources of data:

Col. 1: Sum of California and total of foreign countries.

Col. 2: Compiled from California Crop Reports. These data are not exactly comparable to those shown in tables 1 and 3.

Col. 3: Sum of production for countries for which data are givin in cols. 4, 5, 6, and 7. Persian production, although large, is not included in this table because of lack of reliable data and because its influence on California raisin prices has apparently been negligible. From 15,000 to 30,000 tons of Persian raisins have been exported annually in recent years, almost all being consumed in Russia.

Cols. 4, 6, and 7: Compiled from unofficial estimates largely from reports of the U. S. Dept. Commerce Bur. of Foreign and Domestic Commerce and of the U. S. Dept. Agr. Bur. of Agr. Econ., except the estimates of the authors for Greece and Crete for 1921, 1922 and 1924.

Col. 5: Data for 1921-1928 from Squire, E. C. Australian raisin and currant industry, U. S. Dept. Com. Trade Inform. Bul. 699:6. 1930.

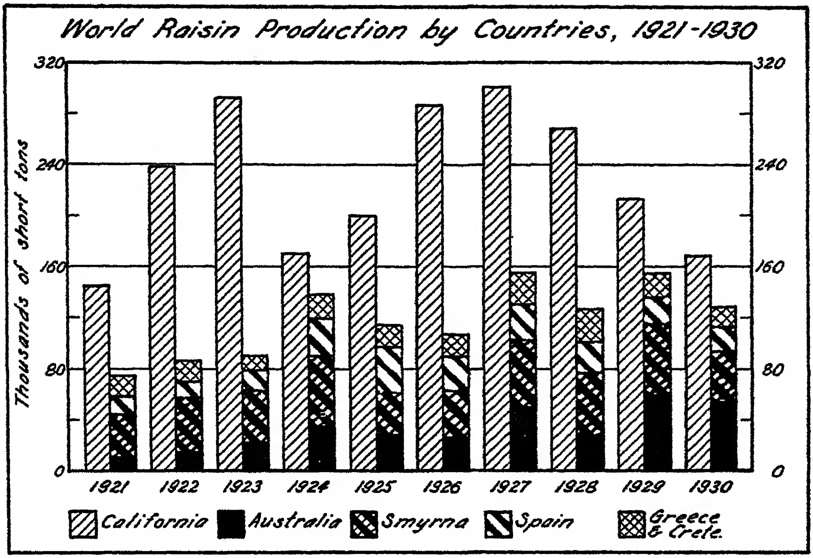

Fig. 3. Data from table 5.

For the purpose of analyzing the factors affecting exports from this state, California f.o.b.-rail export prices have been compared with *United Kingdom prices,* since they are fairly representative of prices prevailing in overseas markets for foreign raisins. Moreover, it is the only country importing raisins from Australia in large quantities and, as figure 3 and tables 5 and 6 show, Australian raisins now constitute a substantial proportion of the world's commercial supplies. *Declared import values per pound* (calculated by dividing the declared import value by the quantity imported) have been used, primarily because no other more satisfactory current price series for California raisins in the United Kingdom was found readily available. The import values per pound also have the merit of being based upon data compiled and issued regularly and promptly each month by a reliable official agency.

In comparing the United Kingdom import values per pound, duty added, of raisins from different countries as shown in table 8, and figure 4, it must be remembered that each is a weighted average of all varieties, types, grades, and packs of raisins imported from each of the designated countries. The relative importance of the different classes of raisins determining the average for each country may differ appreciably. Trade literature gives the impression that a larger proportion of United Kingdom raisin imports from Australia than from California may be of the bleached and dipped types. If this be true

one would expect the price of imports from Australia to average higher, as a whole, than imports from California, since, because of English preference, bleached raisins normally command a higher price than unbleached in that market. Moreover, because of the greater cost of processing bleached and dipped raisins they should in the long run bring higher prices than the natural product, although

TABLE 6

United Kingdom Raisin Imports by Chief Countries of Origin, 1921–1929

Years beginning Sept. 1	Total	United States	Australia	Turkey (Smyrna)	All but United States
	Net weight, short tons				
1921	49,200	7,200	4,800	17,100	42,000
1922	70,600	19,800	9,700	14,000	50,800
1923	62,700	9,700	15,400	21,400	53,000
1924	71,000	13,800	25,700	19,900	57,200
1925	58,500	21,500	12,100	7,500	37,000
1926	74,200	25,000	20,300	13,300	49,200
1927	86,400	33,000	23,400	11,900	53,400
1928	96,800	34,400	35,900	13,000	62,400
1929*	80,100	19,100	35,400	10,200	61,000
	Per cent of total				
1921	100 0	14 6	9.8	34.8	85.4
1922	100 0	28 0	13.7	19.8	72.0
1923	100.0	15.5	24 6	34 1	84.5
1924	100 0	19.4	36.2	28 0	80.6
1925	100 0	36.8	20 7	12.8	63.2
1926	100 0	33.7	27.4	17.9	66.3
1927	100 0	38.2	27 1	13.8	61.8
1928	100 0	35.5	37 1	13.4	64.5
1929*	100 0	23.8	44.2	12.7	76.2

* Preliminary data subject to slight revision.

Source of data:

Basic data compiled from Accounts Relating to Trade and Navigation of the United Kingdom, issued monthly. English hundredweights of 112 pounds converted to nearest hundred short tons.

not necessarily greater net returns to growers. The possible limitations in the comparability of United Kingdom import values per pound of Australian and California raisins, suggest that the differences between the prices of raisins from these two sources may be relative, rather than absolute. Moreover, their comparability will of necessity vary if noncompensating changes in the proportion of high and low-priced raisin imports occur.

Monthly Australian prices weighted by the quantity of California raisins imported into the United Kingdom have been used in getting

an average Australian price for crop years beginning September 1 because it is the quantity of California raisins exported in any given month that presumably is most directly influenced by the foreign prices prevailing during that particular month. Such an average therefore tends to give more weight to the prices of foreign raisins with which California raisins actively compete at any particular time.

TABLE 7

PRODUCTION AND UNITED KINGDOM IMPORTS AND DECLARED IMPORT VALUES PER POUND, DUTY ADDED, OF AUSTRALIAN RAISINS, 1921–1929

Year of harvest or import year beginning April 1	Australian production, sweat-box basis, short tons	United Kingdom imports from Australia: Net packed weight, short tons	United Kingdom imports from Australia: Per cent of Australian production	United Kingdom imports from Australia: Per cent of United Kingdom total imports	Declared import value, duty added, cents per pound	Exchange rate per pound Sterling, in cents
	1	*2*	*3*	*4*	*5*	*6*
1921	9,400	2,282	26	5.6	17.9	397
1922	15,100	5,023	36	7.3	20.4	452
1923	20,900	10,990	57	18.6	16 5	448
1924	33,100	21,934	72	33.7	12 6	454
1925	28,600	18,446	70	27.8	13.2	485
1926	25,100	12,950	56	20.2	14.8	486
1927	49,000	33,541	74	35.5	13.5	487
1928	27,600	14,591	57	19.0	12.1	486
1929	59,000	42,392	77	46 9	9.2	486
1930	59,000*	36.528*	62*	46.9*	8 3*	486

* Preliminary data, subject to slight revision.

Sources of data:

Col. 1: Data given to the nearest hundred tons are for crops harvested in the calendar year indicated, years 1921-1928 from: Squire, E. C., Australian raisin and currant industry, U. S. Dept. Com. Trade Inform. Bul. 699:6, 1930. Data for 1930 are preliminary estimates.

Cols. 2, 4, and 5: Data for years beginning April 1 compiled from Accounts Relating to Trade and Navigation of the United Kingdom, issued monthly. Conversions to cents per pound computed as follows: pounds sterling (£) per English hundredweight divided by 112, times the exchange rates in col. 6. The preferential duties added to the declared import value per pound are for 1921, 1.46 cents; 1922, 1.66 cents; 1923, 1.65 cents; 1924, 1.36 cents; 1925, 0.6 cents. All Australian raisins have entered the United Kingdom duty free since July, 1925, and hence nothing was added to the declared import value per pound for crop years 1926 to date.

Col. 3: Based upon col. 1 and items in col. 2 increased by 7 per cent to convert to an approximate sweat-box equivalent of the net import weight.

Col. 6: Simple average of monthly exchange rates for years beginning April 1, compiled from Federal Reserve Bulletin.

Compared with an Australian price weighted by the quantities of Australian raisins imported into the United Kingdom, it gives heavier weight to Australian prices during the fall and winter months when the majority of California export sales are completed, and much less weight to Australian prices in the following spring and summer when Australian exports are greatest and California's relatively the smallest.

Prices of Australian raisins are shown by figure 4 to be fairly representative of raisins from all foreign countries. The Australian prices, therefore, have been used since they are more readily compiled than the average of all foreign countries and since the price at which each crop of Australian raisins is moving is known for several months in advance of California's harvest. It therefore serves as an important indication of about what prices California may expect to compete with in foreign markets. In using the price of Australia's new crop of raisins in the summer, however, as an indication of the probable level of price competition in the fall, caution must be exercised in years in which the Australian crop is unusually small and the outlook for production in other countries is average or greater. In such years Australian raisins are likely to have a greater price differential over raisins from other foreign countries than usual. For this reason, it is desirable to be particularly well informed regarding the condition of the Turkish crop in judging whether the Australian price during spring and summer months is likely to be representative of the fall harvest of raisins from north of the equator, Turkey being California's next most important competitor, after Australia, in the European raisin market.

Foreign Competition in 1922, 1923, and 1924.—In 1922 the English import price of raisins from Turkey, then the chief foreign competitor of California, was so high as to be detrimental to her volume of sales. With lower prices, therefore, California was able to expand her exports substantially. Although foreign production in 1923 exceeded that of 1922 but slightly (see table 5), prices of foreign raisins were drastically reduced, probably because their sales had dragged so badly the preceding season. The export prices of California raisins were also drastically cut in 1923 and still further reduced in 1924. The import prices of Australian and of other foreign raisins in the United Kingdom, however, were so low in both of these years that they undersold California, reducing exports from this state both in 1923 and 1924 to considerably below the movement in 1922. A decrease in the United Kingdom preferential import duty in August, 1924, was also responsible, to a slight degree, for depressing Australian prices in that market.

Relief Measures for Australian Industry.[9]—Inasmuch as the Australian government had actively encouraged returned soldiers to plant vineyards after the War, it took definite steps to help its raisin industry when the serious prospects of continued low prices

[9] This sketch is based in part on: Bauer, Walter. Australian raisin and currant legislation. An unpublished manuscript in the Giannini Foundation Library.

became evident about 1924. Its first measure was the Act of October 20, 1924, which established a Dried Fruits Export Control Board, the aim of which was to secure optimum returns for the Australian industry, largely by restricting the quantity exported, by establishing a domestic price higher than the possible export level, and by consigning a portion of the domestic retention to industrial (distillery) use. By means of funds from the export levy and from contributions of the Commonwealth Government itself the Board has also carried on a successful publicity compaign for about five years, stimulating the demand for Australian raisins, particularly in the United Kingdom. Similar efforts by the Empire Marketing Board to create 'Empire consciousness' have also helped to increase the demand for Australian raisins in English markets.

Preference for Australian raisins in the Canadian and the United Kingdom markets has also been gained by tariff provisions admitting their raisins free or at greatly reduced rates of duty, whereas other countries (with the partial exception of Greece) pay substantial import duties. Previous to July, 1925, the Australian preference in the United Kingdom was less than half a cent a pound. At that time, however, the preference was increased to 1.5 cents, Australian raisins being admitted duty free (see table 8).

Foreign Competition in 1925 and 1926.—The raisin crops and exports of Australia and Turkey in 1925 and 1926 were considerably smaller than in 1924, and hence they were able to raise their prices in 1925 and 1926. Probably because of the preferential duty and the activities of the Dried Fruit Control Board and the Empire Marketing Board, Australia raised her 1926 prices even higher than Turkey's. California raisin prices in the United Kingdom, however, were lowered both years, so that the import value of her raisins, duty added, averaged nearly 2 cents a pound below that of Australia in 1925 and over 4 cents lower in 1926. This large price differential, together with the relatively small competitive tonnage from Australia and Turkey, enabled California to substantially increase her foreign exports in 1925 and 1926.

Competition from Australia in 1927 and 1928.—Raisin production both in California and in foreign countries was large in 1927, which caused United Kingdom import prices to decline. In spite of the largest raisin crop in her history, however, Australia tried to dispose of the bulk of it in the United Kingdom at a differential over California prices nearly as wide as in 1926. This helped to increase California exports.

In the face of this competition the Australian Export Control Board would probably have been forced to lower their prices in the United Kingdom in the fall of 1927 had not a severe frost on September 24 cut their 1928 crop prospects by one-half. The outlook for a short 1928 Australian crop strengthened the market for all raisins. Although Australia exported unusually large quantities of her 1927 bumper crop at high prices, at the end of the season, April 30, 1928, her London stocks were greater than end-of-season stocks had ever

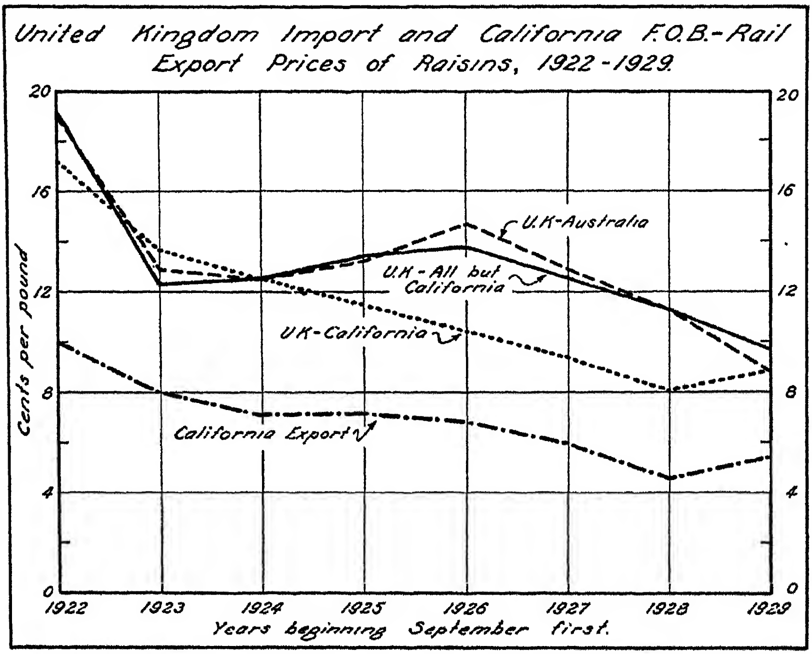

Fig. 4. Data from tables 4 and 8.

been before. Within the year they had risen from about 3,000 to 8,000 short tons. In addition Australian stocks at home were large. Chiefly because of this situation all foreign competitors lowered their prices decidedly.[10] California, however, lowered her export prices nearly as much as her competitors, underselling them by an average of 3 cents during the 1928 crop year. As a result, Australian exports were small and those of California unusually large. The total sales of Australia were only equivalent to her small 1928 crop. This left

[10] Part of the lower average for Australian prices was due to an unusually large proportion of low-grade raisins.

the London stocks on April 30, 1929 still as large as the year before and her inventory at home about 5,000 tons.[11]

The 1929 Decline of Australian Prices.—In addition to large unsold stocks in the United Kingdom, the 1929 Australian crop was a record one. To favor the export of such a crop, the price was dropped about 3 cents in March of that year and had remained between 8 and 9 cents up to February, 1931 (see fig. 5). In spite of the fact that during the year 1929 the Australian price actually averaged lower than that of California, the London stock of Australian raisins in April, 1930, was about 15,000 tons, nearly twice the amount ever before experienced. Moreover, the stocks in Australia amounted to about 10,000 tons.[12] Such a large carryover has helped to keep the 1930 price of Australian raisins at a low level, thereby increasing competition with California raisins.

TABLE 8

UNITED KINGDOM DECLARED IMPORT VALUE PER POUND, DUTY ADDED, OF RAISINS BY CHIEF COUNTRIES OF ORIGIN, 1922–1929

Year beginning Sept. 1	Duty per pound		Exchange rate per £	Import value per pound				
	General	Preferential		United States	Australia	Turkey (Smyrna)	All but U.S.	All countries
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
	cents	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*
1922	2.2	1.7	458.7	17.2	19.0	20.8	19.1	18.5
1923	2.0	1.6	438 1	13.7	12.9	11.6	12.3	12.5
1924	1.4	1.2	465.1	12.6	12 5	12.1	12.5	12.5
1925	1.5	0	485.6	11.5	13.2	15.1	13.4	12.7
1926	1.5	0	485.4	10.5	14.7	13.7	13.8	12.7
1927	1.5	0	487 3	9 4	12.9	11.9	12.5	11.4
1928	1.5	0	485.1	8.1	11.3	10.6	11.3	10.2
1929	1.5	0	486.6	8.8	8.8	9.7	9.7	9.5

Sources of data:

Cols. 1 and 2: Compiled from official sources with conversions to cents per pound as follows: Pounds sterling (£) per English hundredweight divided by 112, times the exchange rates given in col. 3. The general duty in col. 1 is added to the import value of all countries except Greece and British possessions. The preferential duty in col. 2 applies to imports from Australia, South Africa, and other British possessions.

Col. 3: Simple averages of monthly exchange rates for years beginning September 1, compiled from the Federal Reserve Bulletin.

Cols. 4–8: The basic data from which these prices were compiled appear in the Accounts Relating to the Trade and Navigation of the United Kingdom, issued monthly as imports in English hundredweights of 112 pounds and declared import values in English pounds sterling (£). The average prices in col. 5 are computed by weighting the monthly United Kingdom import value per pound of Australian raisins by the quantity of California raisins imported into the United Kingdom during the corresponding months. The prices shown in cols. 7 and 8 include the corresponding prices for Australia shown in col. 5 weighted by the actual quantity of Australian raisins imported into the United Kingdom during the year beginning September 1. The duties added for individual countries and the method of converting to cents per pound are indicated above in the footnote to cols. 1 and 2.

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11 The Fruit World of Australasia 31:200. May 1, 1930.

12 The Fruit World of Australasia 31:200. May 1, 1930.

Relation of California Exports to Prices.—The free hand curve *dd'* in figure 6 is drawn to indicate the average relation between the tonnage and the prices of California raisins exported for the three crop years 1923, 1924, and 1929 in which the United Kingdom import prices of California[13] and of Australian raisins were practically the same. It may be thought of as approximating the overseas demand schedule for California raisins at prices practically the same as those of foreign competitors.

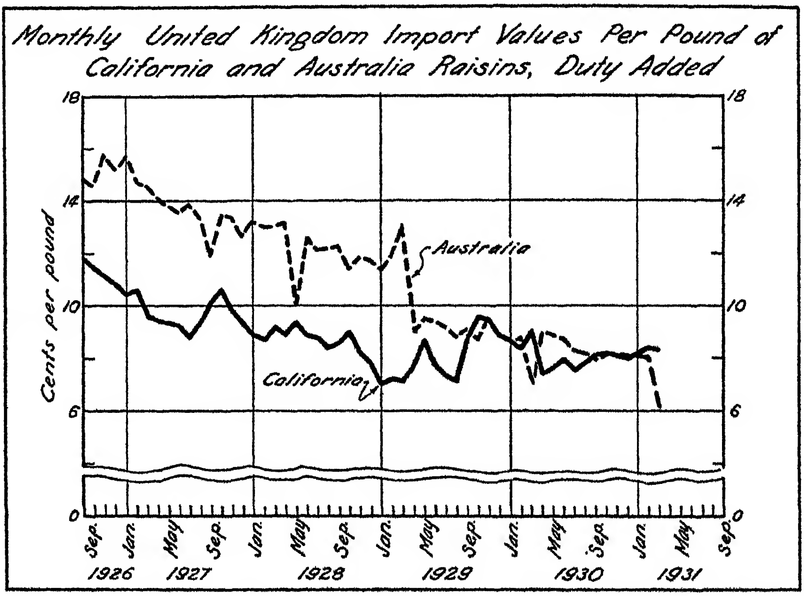

Fig. 5. Data from table 9.

In the other years shown in figure 6, California raisins undercut Australian on the English market by a wide margin, as may be seen in figure 4. This fact appears to account very logically for the increased tonnage of California raisins exported to overseas markets in these other years. Section *B* of figure 6 shows the close relation between these price differentials and the differences between the tonnage of California raisins actually exported in any given year and the tonnage that line *dd'* indicates might have been exported if California and Australian prices had averaged approximately the same.

[13] During the last few years the United Kingdom import price of California raisins, duty added, has been approximately 3.5 cents higher than our f.o.b.-rail export price because of exporting costs and the English import duty.

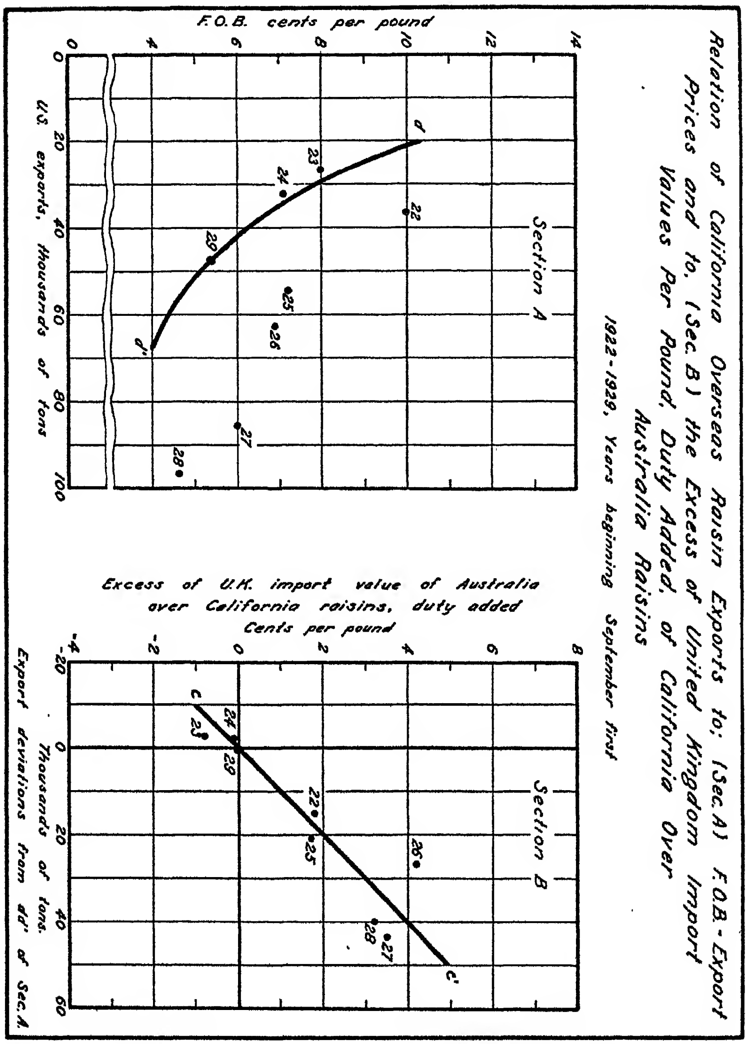

Fig. 6. Data from tables 1, 4, and 8.

These price differentials appear to be a good measure of foreign competition in overseas markets in recent years. The curve *cc′* indicates that annual exports from California have tended to be about 10,000 tons greater for every cent that the Australian price has exceeded California's.

In the years 1923, 1924, and 1929, with little difference between Australian and California prices, competition was keen and California export sales small. The situation, however, was more favorable to California in the years 1925, 1926, 1927, and 1928, in which the Australian price averaged 2 to 4 cents above that of California. As a result, California exported considerably greater quantities than indicated by curve *dd′* in section *A*.

The apparent discrepancy in 1926 was largely due to the fact that Australian production was unusually small and her price somewhat too high to be exactly representative of the competition that California raisins met in foreign markets. Had the smaller price differential between foreign raisins as a whole and California raisins been used in figure 5, the 1926 deviation would be decreased.

TABLE 9

MONTHLY UNITED KINGDOM IMPORT VALUES OF CALIFORNIA AND OF AUSTRALIA RAISINS SINCE SEPTEMBER, 1926, IN CENTS PER POUND, DUTY ADDED

Month	1926–27		1927–28		1928–29		1929–30		1930–31	
	California	Australia	California	Australia	California	Australia	California	Australia	California	Australia
	cents	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*	*cents*
September	11.8	14.8	10.1	11.9	8.6	12.3	8 8	9.1	8.1	7.9
October	11.4	14.5	10.6	13.5	9.0	11.4	9.6	8.7	8.2	8.1
November	11.1	15.8	9.8	13.4	8.2	11 8	9.5	9.7	8 3	8.2
December	10 8	15.2	9.3	12.6	7.8	11.7	8.8	8.9	8.0	8.1
January	10.4	15 7	8.9	13.2	7.0	11.4	8.7	8.6	8.2	8 1
February	10.6	14 7	8.7	13 0	7.2	11.9	8.4	8.8	8 4	8.1
March	9.6	14.6	9.2	13.0	7.1	13.1	9.2	6.9	8.3	6.1
April	9.5	14.1	8.9	13.2	7.7	9 0	7.4	9.0	....	....
May	9.4	13.8	9.4	10.0	8 7	9.5	7.6	8.9	....	....
June	9.3	13.6	8.9	12.6	7.7	9.4	7.9	8 7	....	....
July	8.8	13.9	8.8	12.1	8.3	9.2	7.5	8.3	....	....
August	9.3	13 3	8.4	12.2	8.1	8.8	7.8	8 1	....	....
Weighted Ave.	**10.5**	**13.8**	**9.3**	**12.8**	**8.2**	**9 6**	**8.8**	**8.7**	....	....
Exchange rate	485.4	485 4	487.3	487.3	485.1	485.1	486.6	486.6	486.6	486.6

Source of data:

The basic data from which these prices were compiled appear in the Monthly Accounts relating to Trade and Navigation of the United Kingdom as imports in English hundredweights of 112 pounds and declared import values in English pounds Sterling (£). The import value in cents per pound is computed as follows: £ per hundredweight divided by 112 times the simple average of monthly exchange rates for years beginning September 1 as shown in the bottom line of the table. The duty of 1.5 cents a pound was added to get the California price as given. Australian raisins have been admitted duty free since 1925. The annual prices are weighted averages.

OTHER CONSIDERATIONS

Although the greater part of the variations that have occurred in the crop-year sales and prices of California raisins during the last nine years have been accounted for in this analysis, there are probably several other factors, mostly minor, that have exerted some influence, such as trend in demand, competition from currants[14] and other fruits, variations in variety, type, quality, and style of packages, advance or decline in prices during the year, the lag of retail prices, changes in the general price level, and the psychological attitude of the trade as affected by facts or lack of dependable facts.

The foregoing analysis explains only what has occurred. It may not explain what will occur, as attendant conditions may be very different in the future than they were during the period upon which this analysis is based.[15] However, it should give the industry a better basis than previously has been available for judging the probable price at which a given supply of California raisins may be sold in the future. In using it as a partial basis for deciding what is probably the best raisin-marketing policy to pursue in any particular year, a trained judgment that can ordinarily be acquired only as the result of close first-hand acquaintance with the business of marketing raisins obviously is essential, coupled with an intimate understanding of just what the current situation is and what business conditions are likely to exist during the marketing season.

APPLICATION

Upon the basis of curves in figures 2 and 6, the schedule of prices and sales of California raisins given in table 10 has been prepared to illustrate the method of using this analysis. It shows the approximate relation between the tonnage of California raisin sales and the f.o.b.-rail prices, with domestic demand conditions adverse as indicated by the lower curve in figure 2 (at the level of demand in 1929)

[14] The relation between raisin prices and currant supplies and prices that one might reasonably expect seems to be obscured by the fact that in many years California and world raisin production have both been large when currant production was small, and vice versa.

[15] For example, if large quantities of low-priced raisins from Russia were to be dumped on European markets, as rumored in the dried-fruit trade. See, for example, Taylor, Alonzo E. Cooperate or bust. *Country Gentleman* 50 (6):4. June, 1931.

and also moderately favorable as indicated by the middle curve in the same figure. The foreign sales are based on f.o.b.-rail prices with an allowance of a 3.5-cent margin to approximate the equivalent United Kingdom import value per pound, duty added, as given in column 6. Moreover, the relation shown between the price and the tonnage exported assumes the same level of United Kingdom average import values per pound, duty added, for California, Australia, and other raisins. As shown by section *B* of figure 6, in years in which the United Kingdom import value, duty added, of California raisins has differed from that of raisins from other countries, an allowance of about 10,000 tons for each cent in the price differential has been necessary.

TABLE 10

APPROXIMATE DOMESTIC AND FOREIGN SALES OF CALIFORNIA RAISINS UNDER ADVERSE AND MODERATELY FAVORABLE DEMAND CONDITIONS IN THE DOMESTIC MARKET AND UNDER FOREIGN COMPETITIVE CONDITIONS IN WHICH THE UNITED KINGDOM IMPORT PRICE, DUTY ADDED, IS THE SAME FOR BOTH AUSTRALIA AND CALIFORNIA

Domestic sales			Foreign sales		
Quantity	F.o.b.-rail price per pound with demand conditions		Quantity	California price per pound with United Kingdom import price of Australian and California raisins same	
	Adverse	Moderately favorable		F.o.b.-rail price	Equivalent United Kingdom import price, duty added
1	*2*	*3*	*4*	*5*	*6*
tons	*cents*	*cents*	*tons*	*cents*	*cents*
187,000	3.5	5.4	66,000	4 0	7 5
180,000	4.0	6.0	58,000	4.5	8 0
173,000	4 5	6.7	52,000	5.0	8 5
167,000	5.0	7.3	47,000	5.5	9.0
161,000	5.5	8 0	42,000	6 0	9 5
156,000	6.0	8.5	38,000	6.5	10.0

Source of data:

Col. 2: Based upon the lowest curve (for 1929) in figure 2.

Col. 3: Based upon the middle curve in figure 2.

Cols. 4 and 5: Based upon section *A* of figure 6.

Col. 6: Items in col. 5 plus 3.5 cents per pound.

The table indicates for example, that 167,000 tons of California raisins might be sold in the domestic market at an f.o.b. price of 5 cents under economic conditions like those in 1929. On the other hand, under better conditions of demand, such as the data in column 3 are based upon, the same quantity could be sold at a price of about 7.3 cents.

With the United Kingdom import value per pound the same for both California and Australian raisins, California export sales at a 5-cent f.o.b.-rail price, equivalent to about an 8.5-cent United Kingdom import value, duty added, would appear to be about 52,000 tons. When prices of Australian raisins have exceeded California prices in that market, California's exports have tended to increase about 10,000 tons for each cent of differential as indicated in section *B* of figure 6.

NEEDED CURRENT STATISTICAL DATA

To use effectively the methods and results of this analysis as a partial basis for a marketing and sales policy, the industry must have available certain data on supply at the beginning of the season. Furthermore, in order to check upon the the results of the policy adopted and to modify it, if needs be, during the season, current and cumulated data on both prices and quantities sold or shipped are needed. The more important of these statistical data are:

1. *Estimates of California Raisin Production.*—Since it is necessary for buyers and sellers to decide on price and marketing policies early in the season, estimates of the probable raisin output are needed by September 1 or earlier. Preliminary official estimates by the California Crop Reporting Service of the tonnage of raisin grapes dried have not been available in the past until sometime in December. However, preliminary estimates of probable production of California raisin grapes are made by September. The probable tonnage that will be dried is the difference between this estimate of raisin-grape production and the quantities shipped fresh and not harvested. The probable total of fresh raisin-grape shipments is ordinarily not known with any considerable degree of precision until well into October. However, some help in forecasting this may be secured from market information and from the better informed of the shippers and the trade. The difference between the prevailing prices offered the grower for his raisins early in the season by packers, and the prices received for fresh raisin-grape shipments exerts an appreciable influence on the tonnage diverted for drying or for shipping fresh. Some basis for determining the probable effect of these price differentials on utilization are indicated in the accompanying paper.[16]

2. *Carryover of Raisins in California on September 1.*—Carryover plus estimates of production indicate supplies available for sale dur-

[16] Mallory, L. D., S. R. Smith, and S. W. Shear. Factors affecting annual price of California fresh grapes, 1921–1929. Hilgardia, 6:101–130. 1931.

ing the current marketing season. In the past there have been no reliable data available on the stocks of raisins in California in the hands of the packers and Sun-Maid Raisin Growers Association on September 1. However, the Dried Fruit Association has secured this information from its members for the fall of 1930. If similar data are secured in the future and released as soon after September 1 as possible, the determination of sound price and marketing policies for raisins will be greatly facilitated.

3. *Tonnage Sold Monthly, Domestic and Overseas Separately.*—Quantities sold currently during the marketing season, along with the actual sale prices, and a knowledge of seasonal variations of both in past years are essential to judging the results of the marketing and price policy being pursued and in deciding whether to modify it or not and if so, how. Moreover, with the help of such monthly data, current stocks of raisins in California can be approximated in the absence of better data on carryover.

Total monthly shipments of California raisins can be compiled fairly accurately from the following series of data, each of which, at present, must be secured from different agencies. They could be rendered more readily available if assembled and released to the industry monthly by a single agency.

(*a*) Monthly shipments of California raisins from the ports of this country to overseas countries and to Canada are available in the Monthly Summary of Foreign Commerce of the United States.

(*b*) No similar official data on monthly shipments to domestic markets are available. However, monthly shipments by rail from California are available for raisins and for other dried fruits separately. These are released monthly in mimeographed form by the Dried Fruit Association of California, based upon reports received from each railroad. A number of the larger packers also receive these reports direct from the railroads.

(*c*) In addition, direct exports from San Francisco and Los Angeles by water to foreign countries are available in the monthly blotters of the United States Department of Commerce, Bureau of Foreign and Domestic Commerce, usually published in various trade papers.[17] Intercoastal shipments from California by steamer to domestic ports are not readily available, although the individual steamship companies send monthly reports covering these data to a few of the packers.

[17]For example in the California Fruit News and the Western Canner and Packer.

(*d*) The only quantities not included in the monthly completed sales of California raisins, shown by rail shipments out of the state plus direct exports by water to foreign countries and domestic intercoastal shipments, are the small quantities sold and consumed in California. These, however, are a relatively small proportion of the total and can be estimated fairly satisfactorily on a per-capita basis comparable to consumption in the rest of the United States.

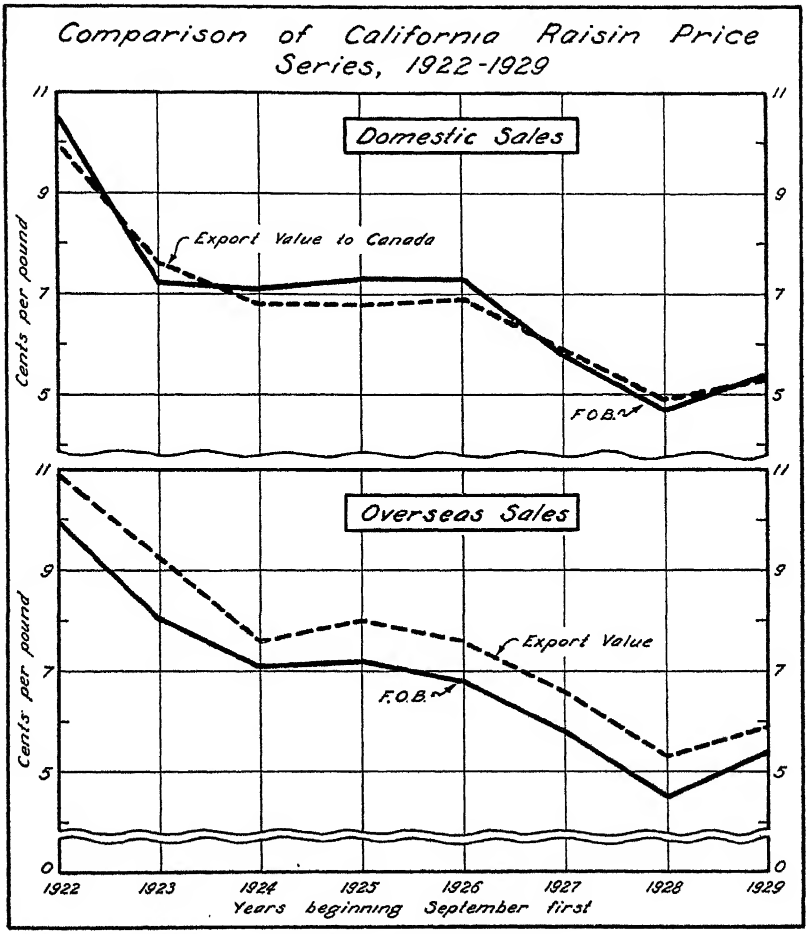

Fig. 7. Data from table 11.

4. *Average Prices of Current Season's Sales to Overseas Markets.* —Comparison of various raisin-price series that are available indicates that the average declared export values per pound of raisins

exported from the United States to all overseas markets or to the United Kingdom alone are about the best relative indicators of actual prices of California sales to overseas markets. However, study of export values per pound for direct exports from San Francisco may prove them to be even better than those based upon total exports from all United States ports. Declared export prices are easily computed by dividing the quantity exported to overseas markets into the declared values of such exports. Data on the quantity and declared values of exports are available monthly in the Monthly Summary of Foreign Commerce of the United States and can easily be cumulated as the basis of a weighted average for the crop year to date.

Comparison of these declared export values per pound for years beginning September 1, 1922–1929, with the actual f.o.b.-rail prices of overseas sales reported by Sun-Maid Raisin Growers Association and the packers for the corresponding crop years, as shown in table 11, shows that the two have been closely correlated since 1924, the period during which the f.o.b. prices were most completely and accurately reported. The declared export values per pound naturally should be above the f.o.b.-rail prices to cover additional items of expense involved in exporting.

Comparison of United Kingdom import values per pound, duty added, of California raisins (see col. 8, table 11, page 99), with the f.o.b.-rail price of overseas sales shown in figure 7, indicates that in the last four years there has been a rather consistent difference of about 3.5 cents between these two series. The import values into the United Kingdom therefore also appear to have been a fairly good index of California f.o.b.-rail prices of overseas sales in recent years.

5. *Average Prices of Current Season's Sales to Domestic Market.* —Comparison of the domestic f.o.b.-rail price series reported by the packers and the Sun-Maid Raisin Growers Association has been made with a number of other readily available current price series and found to be rather closely correlated with them. Comparison of columns 1 and 2 in table 11 shows that in recent years there has been very little difference between the f.o.b.-rail domestic price of California raisins and the declared export values per pound of California exports to Canada. This relation appears quite logical. Since the monthly and cumulated quantity and declared values of California exports of raisins to Canada are readily available in the Monthly Summary of Foreign Commerce of the United States, the declared import values per pound based upon them are probably the most convenient indicator of current f.o.b.-rail domestic prices of California raisins now available.

Season's averages of monthly quotations from the New York Journal of Commerce for California seeded and seedless raisins have shown a fairly close relation to domestic f.o.b. prices since 1922 (see table 11) and also to declared values per pound of overseas exports.

TABLE 11

COMPARISON OF DIFFERENT SERIES OF CALIFORNIA RAISIN PRICES IN CENTS PER POUND, 1922–1929

Crop year	Domestic sales				Overseas sales			
	F.o.b.-rail California	Export value to Canada	N. Y. wholesale	U. S. retail	F.o.b.-rail California	Export value	Export value to United Kingdom	United Kingdom import value, duty added
	1	2	3	4	5	6	7	8
1922	10.5	10.0	10.5	18.4	10 0	10.9	10.5	17 2
1923	7 2	7.6	7.8	15.8	8.0	9.3	8.8	13.7
1924	7.1	6.8	7.7	14.6	7.1	7.6	7.5	12.6
1925	7.3	6.8	7.5	14.6	7.2	8.0	8.0	11.5
1926	7.3	6.9	7.8	14.4	6.8	7.6	7.5	10.5
1927	5.8	5.9	7.0	13.7	5.8	6.6	6.6	9.4
1928	4.7	4.9	5.0	11.8	4.5	5.3	5.1	8.1
1929	5.4	5.3	6.0	12.1	5.4	5.9	5.8	8.8

Sources of data:

Col. 1: from col. 4, table 4, page 80.

Cols. 2, 6, 7: United States exports of California raisins for years beginning September 1, declared export value divided by pounds exported. Basic data compiled from Monthly Summary of Foreign Commerce of the United States. Col. 2 includes exports to Canada only; col. 6 exports to all other countries, except Canada, col. 7 exports to the United Kingdom only.

Col. 3: Based upon monthly quotations nearest the end of each month of California bulk seeded Muscats and bulk Thompson Seedless raisins on the New York wholesale market compiled from the last issue of each month of the New York Journal of Commerce. An average for the 12 months beginning September 1 was computed separately for seeded and seedless by weighting by the monthly shipments of California raisins. The combined average of these two annual prices computed by weighting by the percentage of California production by varieties shown in cols. 2 and 3 of table 2, page 75 are the final averages given above.

Col. 4: Simple average of monthly United States retail price of raisins for years beginning October 1, compiled from the Monthly Retail Prices of the U. S. Bur. of Labor Statistics.

Col. 5: From col. 5, table 4, page 80.

Col. 8: From col. 4, table 8, page 89.

Table 11 shows that there has also been a rather consistent relation between the retail price of raisins in the United States and the domestic f.o.b. price, the former being rather consistently 7 to 8 cents higher than the domestic California f.o.b. price and the New York wholesale price.

6. *United Kingdom Declared Import Values per Pound of Raisins—California, Australia, and Other Countries—Monthly and by Crop Years.*—These prices are based on the data on monthly quantity and declared import values readily available in the Monthly Accounts Relating to the Trade and Navigation of the United Kingdom as indicated in the footnote to table 8, page 89.

ACKNOWLEDGMENTS

This study was made under the guidance of Professor H. R. Tolley, Director of the Giannini Foundation. It has been possible to present many of the most important California data included in the analysis only because of the generous cooperation of the Sun-Maid Raisin Growers Association and of the independent packers acting through the Dried Fruit Association of California. Generous assistance in supplying data and counsel were given by Dr. Holbrook Working and Dr. Alonzo E. Taylor of the Food Research Institute at Stanford University, Dr. E. W. Gaumnitz of the Division of Markets of the California State Department of Agriculture, Professor Donald Sham of Santa Clara University, and Messrs. L. A. Wheeler of the United States Department of Agriculture Bureau of Agricultural Economics, R. S. Hollingshead of the United States Department of Commerce Bureau of Foreign and Domestic Commerce, M. E. Brooding of the California Packing Corporation, K. R. Richardson, formerly with the Sun-Maid Raisin Growers Association, P. Malloch, Manager of Irymple Packing Pty., Australia, Dr. N. J. Silberling of the University of California, and Dr. H. R. Wellman, Mr. E. W. Braun, and Dr. W. Bauer of the College of Agriculture.

FACTORS AFFECTING ANNUAL PRICES OF CALIFORNIA FRESH GRAPES, 1921-1929[1]

L. D. MALLORY[2], S. R. SMITH[3], AND S. W. SHEAR[4]

This paper presents the results of an analysis designed to discover and measure the influence of the major factors that have affected the season's price of each class of California fresh grapes—table, black-juice, and white-juice.

The total quantity of California grapes produced in any one year is determined by the bearing acreage, the environmental conditions of growth in that year, and the care expended in their culture. During any one harvesting season, therefore, the total available supply of grapes is not subject to great change. Because the supply of fresh grapes for any given season is relatively fixed it is primarily price-determining rather than price-determined. However, the two-way usage of raisin grapes has some effect upon the quantity of that class of grapes marketed fresh. Raisins take a large portion of the crop, and the relative profitableness of shipping fresh or of drying into raisins influences the amounts utilized in one way or the other. The two uses, however, tend toward equality of returns. With a relatively fixed supply for any given year, changes in price other than those accounted for by year-to-year changes in fresh shipments, therefore, are the result of factors influencing demand. Because of this fact, a large part of this study is devoted to factors which have influenced the demand for fresh grapes.[5]

[1] Paper No. 21, The Giannini Foundation of Agricultural Economics. This study was made with the financial cooperation of the Federal Farm Board.

[2] Research Assistant in Agricultural Economics.

[3] Temporary Research Assistant in the Federal Farm Board.

[4] Associate Agricultural Economist in the Experiment Station and Associate Agricultural Economist on the Giannini Foundation.

[5] An excellent discussion of the relation of statistical analysis to the laws of price will be found in: Ezekiel, Mordecai. Statistical analysis and the 'laws' of price. Quar. Jour. of Econ. 42:199–227. Feb. 1928.

Some discussion of factors affecting California grape prices will be found in the following: Shear, S. W., and H. F. Gould. Economic status of the grape industry. California Agr. Exp. Sta. Bul. 429:81–94. 1927. *(Continued on page 102)*

Were the schedule of demand for California grapes to remain the same over a period of years, it would be possible from data on annual prices and shipments to construct a schedule of prices at which different numbers of carloads could be sold. Explanation of prices and forecasting of season's average prices from shipments would thus be made easy. However, the problem is far from being simple because the demand for the various classes of grapes changes from year to year.

DATA USED

It was necessary to base this analysis on data covering a period of only nine years. Data for years previous to 1921 are limited both as to scope and accuracy. Moreover, the effects of the very abnormal influences of the World War upon prices and other economic conditions for the years just previous to 1921 made the inclusion of data for earlier years inadvisable.

Even as it is, the rapid changes that have occurred in the California grape industry since 1920 have introduced decisive trends and other changes into the data, the effects of which have seriously complicated the analysis. Grape prices, along with other fruit prices, remained high for a year or two longer than the price of staple farm products immediately after the War. Eastern demand for juice grapes was also stimulated by prohibition. Largely as a result of these two facts, grape plantings increased rapidly in California for several years after 1921, with the consequence that production and shipments have followed a rapid upward trend accompanied by drastic declines in the price of all classes of grapes.

Because the greater part of California fresh grapes has been consumed in eastern cities, prices accurately representing actual sales in these markets were desired. For this reason, farm prices were not used nor, chiefly for the same reason, were f.o.b. prices. Moreover, for the years included in the analysis, f.o.b. prices were not quoted

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Stover, H. J. Relation of the production of grapes in western New York and in California to prices. N. Y. S. College Agr., Cornell Univ., Farm Economics No. 59, p. 1111–1113. June, 1929.

Stover concludes that "The multiple correlation between (annual data 1910–1926 on) the purchasing power of the price (i.e. adjusted price) of table grapes in California (X_1), the production of grapes in western New York (X_2), and the production of table grapes in California (X_3): $R_{1.23} = 0.768 \pm 0.067$, indicates that the production of grapes in the two areas accounts for 59 per cent of the factors determining the price received in California. Of this amount, 10 per cent is due to western New York production and 49 per cent to California production. (Note that relatively few California table grapes were unharvested before 1927.)

with sufficient regularity to constitute complete series, their manner of collection was not sufficiently accurate, and the quantities sold at the various prices were not available.

An accurate measure of price could have been obtained from the sales on the eleven auction markets.[6] Comparison showed, however, that New York delivered-auction prices and the average for the eleven delivered-auction markets differed relatively little. The New York market area receives approximately 20 per cent of the interstate movement of California grapes. Moreover, it is in the same diversion zone as Boston, Baltimore, Pittsburgh, and Philadelphia. Hence, New York auction prices were chosen as representative of eastern sales. Using prices for this one market also obviated the necessity of taking into account varying lengths of time required for transportation from California to different markets when studying the movement of prices during a season, making possible the use of uniform lags between time of shipment and prices upon arrival.

As a measure of the annual quantities of each class of California fresh grapes bought at prices equivalent to the annual New York delivered-auction prices, annual rail shipments (inter and intrastate) of each class of grapes were used. Local truck movement and some small additional tonnage consumed in California is, of course, excluded from rail movement, but the total is relatively small, is difficult to estimate accurately, and probably has influenced eastern prices but little, if at all.

About the only other part of California grape production excluded from rail shipments that may have influenced eastern prices of fresh grapes has been the quantities left unharvested in a number of recent years and the tonnage of raisin grapes that might be diverted from drying to swell the fresh movement. Although the unharvested tonnage may have had some influence psychologically in depressing eastern prices, largely because of the uncertainty of whether some of it might be shipped, the effect has apparently been too small to be appreciable or else has been obscured by other factors. The option of raisin-grape growers of drying or of shipping their grapes fresh has had an appreciable bearing upon fresh Muscat shipments, as discussed later, but has been exercised but little in the case of Thompson Seedless and Sultana.

An added advantage of using data on rail shipments is that they provide comparable supply data as a basis for studying seasonal

[6] Baltimore, Boston, Chicago, Cincinnati, Cleveland, Detroit, Minneapolis, New York, Philadelphia, Pittsburgh, St. Louis.

variation in prices. Additional data used on current movement were arrivals, track holdings, and unloads. Data on unloads, however, were unavailable for 1925 and 1926, and hence the volume of delivered-auction sales was substituted as a measure of current rate of movement into consumption.

TABLE GRAPES

As shown by figure 1, variations in the supply of California table grapes account for nearly all the year-to-year changes in adjusted seasonal average[7] prices for the nine years 1921 through 1929.[8] As California shipments have increased (fig. 1 and table 1), New York delivered-auction prices have declined, and vice versa. The regression line *dd′*, fitted free-hand to the points of the scatter diagram, shows the close relation normally existing between prices and shipments and indicates about how much of a change in price might normally be expected from a given change in table-grape shipments. The demand indicated by this curve is somewhat inelastic. Hence the total value of grapes sold in eastern markets tends to be smaller when annual shipments are heavy than when they are light. With shipments of from about 25,000 to 31,000 carloads of table grapes, elasticity of demand varies from somewhat more than 0.8 with the lighter shipments and higher prices to somewhat less than 0.7 with the heavier shipments and lower prices. The demand appears to be most elastic when supplies are small and prices relatively high.[9].

The regression line in figure 1 may be used to estimate the adjusted price at which any given number of carloads of California table grapes may be expected to sell during a marketing season. The

[7] Variations in the general level of prices were relatively small during the period 1921–1929 and the variations in grape prices associated with them were too small to be important. However, the probability of future substantial changes in the general price level which might appreciably influence grape prices, led to the decision to adjust actual prices during the period studied. The Bureau of Labor Statistics all-commodity wholesale price index was used in making these adjustments.

[8] This study was completed before adequate data on the marketing of the 1930 crop were available, and hence they have not been included in the present analysis. However, the preliminary data available show that table-grape prices in 1930 were very closely in line with the regression curve in figure 1. About 26,800 carloads were shipped and the average New York delivered-auction price was about $95 a ton actual or about $110 adjusted.

[9] For a comparison with the elasticity of other classes of California grapes see page 111 for black-juice, page 121 for fresh Muscats. The elasticity of domestic demand for raisins is given in the accompanying paper: Shear, S. W. and R. M. Howe, Factors affecting California raisin sales and prices, 1922–1929. Hilgardia 6:78. 1931.

estimated adjusted price can then be converted to an estimated unadjusted price by multiplying by an estimate of the all-commodity wholesale price index for the current calendar year and pointing off two decimal places.

Obviously the relation shown in figure 1 is not perfect, for all the points in the scatter do not fall exactly upon the regression line. Other factors than shipments, therefore, have apparently affected eastern prices of California table grapes.

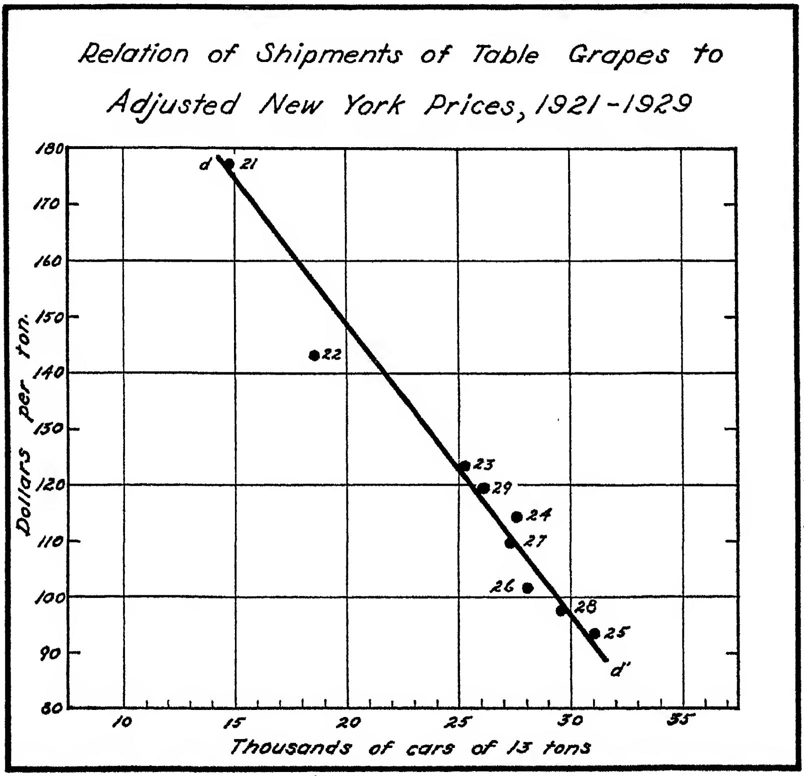

Fig. 1. Data from table 1.

Table grapes constitute on the average only some 30 per cent of the annual shipments of all California fresh grapes. The question, therefore, arises as to whether or not the supplies of other classes of grapes (black-juice and white-juice) have had an effect upon the price of table grapes. Superficially it seems that they would have had. However, although some Malaga and a few Tokay and Thompson Seedless have been sold as juice stock, analysis indicates that the small

tonnage of fresh grape shipments having a two-way use has had little, if any, influence on the annual average price of California table grapes. The plotting of the deviations of price from the regression line in figure 1 against shipments of black-juice grapes and Muscats (severally, combined, and in conjunction with other factors) failed to show that these factors had a measurable influence. Apparently the price of table grapes has been little affected by changes in the proportion of total California shipments consisting of black-juice and white-juice grapes. Such a conclusion appears logical when an examination is made of the use of the different variety classes, of the marketing channels, and of the consumers of the different grapes.

TABLE 1

ANNUAL SHIPMENTS AND NEW YORK DELIVERED-AUCTION PRICES OF CALIFORNIA TABLE GRAPES, AND GRAPE SHIPMENTS OF OTHER STATES, 1921–1929

Crop year	Shipments		Price per ton	
	California	Other states	Unadjusted	Adjusted
	1	*2*	*3*	*4*
	carloads	*carloads*	*dollars*	*dollars*
1921	14,800	4,473	172.80	177.05
1922	18,600	15,967	138.40	143.12
1923	25,240	9,988	124.00	123.26
1924	27,600	12,238	112 00	114.17
1925	31.100	5,812	96 80	93.53
1926	28,100	14,732	101.60	101.60
1927	27,300	6,752	104 80	109.85
1928	29,600	8,611	95.40	97.65
1929	26,100	6,897	115.20	119.38

Sources of data:

Col. 1: Total shipments, to the nearest hundred, both inter and intrastate, of all table-grape varieties—Malaga, Tokay, Emperor, Cornichon, and Almeria are the chief ones, and also of Thompson Seedless—in carloads of approximately 13 tons net of grapes. Data for 1928 and 1929 increased 6 per cent to allow for heavier loadings per car. Data for 1921–1926 from reference 1, *p. 46*. Note—Hereafter references used in sources of data will be numbered according to literature cited on pages 129 and 130). Data for 1927–1929 estimated by applying the variety percentages of interstate shipments to total California shipments including intrastate movement. Basic data for 1927 from reference 2, *p. 10-12* and for 1928 and 1929 from references 3 and 4.

Col. 2: Carlot shipments from states other than California. Data for 1921-1923 from reference 5, *p. 37-41;* for 1924–1926 from reference 6, *p. 188* and for 1927–1929 from U. S. Dept. Agr. mimeographed summaries, Monthly Carlot Shipments of Grapes.

Col. 3: Weighted average prices of New York delivered-auction sales of Tokay, Malaga, Cornichon, and Thompson Seedless varieties, in lugs and crates, through the first or second week of November. Converted to price per ton at the rate of 80 packages to the ton. Averages based on daily data as originally reported in the New York Fruit Reporter and summarized for 1924 in reference 12, *p. 33-35:* for 1925, 1926, and 1927 in reference 2, *p. 80-96;* for 1298, in reference 9, *p. 47-94;* and for 1929 in reference 4.

Col. 4: Prices adjusted to 1926 base by use of U. S. Dept. Labor Bureau of Labor Statistics all-commodity index of wholesale prices for calendar years.

Table grapes ordinarily reach the ultimate consumer through retail stores, hotels, restaurants, etc., in very small lots for dessert purposes or for eating out of hand. Juice grapes, on the other hand, are seldom sold in lots of 5 or 10 pounds but reach the purchaser or consumer in lots of several lugs. This is because fairly large quantities are necessary for satisfactory results in the processing of a 'batch' of juice. A considerable portion of the juice grapes are sold in rather large quantities to people of foreign nativity, who, through custom or habit, demand a juice beverage for drinking purposes or as a part of their diet. This discrimination in consumptive uses of table grapes and of juice grapes probably largely accounts for the lack of price relation between the variety classes.

Unlike California juice grapes, the American type of slip-skin grapes of the *labrusca* species grown commercially in large quantities in eastern states is largely used for table consumption. Shipments of these grapes might, therefore, be expected to affect the demand for California table grapes in eastern markets. Plotting of the price residuals of figure 1 against total carlot shipments of grapes other than California (see table 1) indicates that shipments of eastern grapes have had some influence upon the price of California table grapes. In the scatter diagram, figure 1, it will be seen that the points for the years 1922 and 1926 fall below the line, indicating a smaller average price than would have been expected from a normal relation. In each of these years shipments of eastern grapes were particularly large, being 15,967 carloads in 1922 and 14,732 carloads in 1926. However, the volume of eastern grape shipments apparently does not usually have much influence on the price of California table grapes. The two years noted are the only ones out of the nine in which a decided effect can be observed. It was sufficiently important in those years, however, to warrant its being taken into account in any possible predictions.

There are some indications that the earliness or lateness of the bulk of market arrivals of eastern-grown grapes in relation to California arrivals may also affect the influence of season's total supplies of *lubrusca* grapes on California table-grape prices.

Because outstanding differences in the seasonal variation in shipments and prices might logically be expected to affect the relation of total season's shipments to season's average prices of California table grapes, such an analysis was made. The results were somewhat disappointing. In the analysis of average annual prices the relation between season's total shipments and average annual prices was marked,

but the relation between weekly shipments and price provided results of only meager value in judging the course of prices from current supplies. It was found, however, that during seasons when shipments from California to eastern markets have assumed a fairly normal, orderly, and uniform movement, excluding periods in which there were abnormal factors such as a truckmen's strike, no major fluctuations have taken place. When shipments have moved East irregularly and large track holdings have accumulated in eastern markets, violent price fluctuations have usually taken place. The attempts of various California shipping organizations in recent years to avoid market gluts appear to have improved the seasonal movement of prices, thereby raising the average price for the season. The study of seasonal variations and fluctuations has emphasized the fact that current weekly prices in eastern markets are affected not only by current shipments but also, during the greater part of the shipping season, by total shipments expected during the whole season. This observation also applies to juice grapes.

The response of weekly prices to shipments during the four years 1926–1929 may be observed in figure 2. The year 1926 is a good example of great fluctuations in weekly prices due to irregular shipments. In contrast, during 1928 and 1929 shipments maintained a uniform flow and weekly prices were fairly uniform, with the exception of the portion of October, 1929, during which the truckmen's strike in New York City occurred.

Unfortunately, lack of appropriate data precluded statistical analysis of the influence of quality upon eastern prices of California table grapes, for there can be no doubt that quality has a substantial effect on prices.[10] No accurate measure of the quality of grapes has been devised and the fragmentary information available is quite inadequate as a basis for statistical analysis. Attractive appearance—well-formed bunches of sound berries free from blemish—together with good flavor and high sugar content, are known to stimulate buying, while poor quality causes consumers to buy only at lower prices or else to turn to substitute fruits if available at attractive prices. Table grapes of poor quality are frequently sold for juice purposes at prices so low that they appreciably reduce the average annual price of table-grape varieties as a group.

[10] According to a letter of June 11, 1930, from Mr. Earl R. French of the New York Food Marketing Research Council to the authors, a number of men in the fruit trade and in marketing research in New York City whom he interviewed expressed the opinion that next to quantity of the market supply, quality is perhaps the most important factor affecting California grape prices in eastern markets.

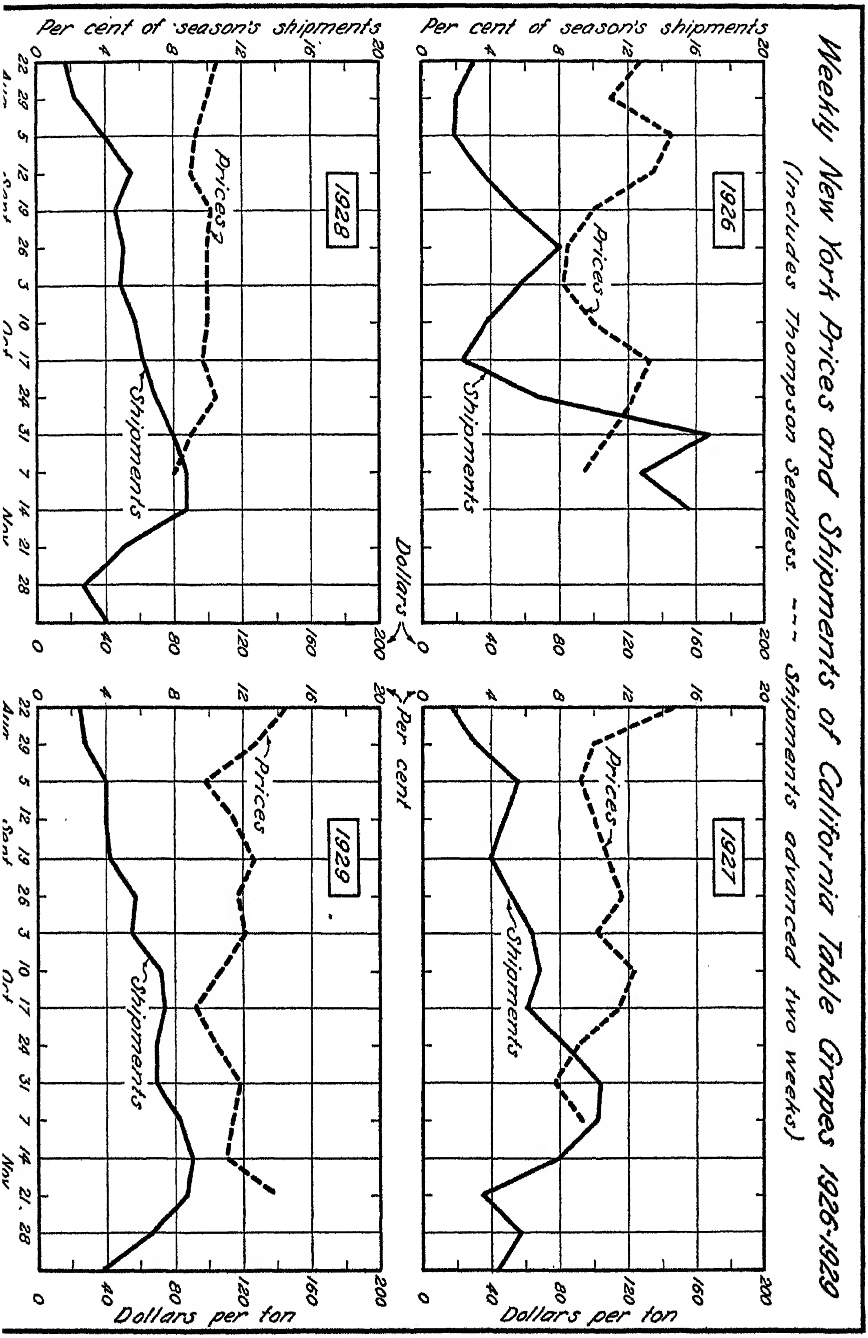
Weekly New York Prices and Shipments of California Table Grapes 1926-1929
(Includes Thompson Seedless. --- Shipments advanced two weeks)
1926
1927
1928
1929
Prices
Shipments
Per cent of season's shipments
Dollars per ton
Dollars
Per cent

TABLE 2

WEEKLY NEW YORK DELIVERED-AUCTION PRICES AND INTERSTATE SHIPMENTS IN CARLOADS AND IN PER CENT OF SEASON'S TOTAL, OF CALIFORNIA TABLE GRAPES, 1926–1929

Week endings (as of 1926)	Price, dollars per ton	Shipments		Price, dollars per ton	Shipments	
		Carloads	Per cent of season's total		Carloads	Per cent of season's total
	1	*2*	*3*	*4*	*5*	*6*
	1926			1927		
Season's total	..........*	20,340†	100.00†	....	26,711†	100.00†
Aug. 7	87.20	618	3.04	248.80	476	1.78
Aug. 14	119.20	404	1.99	166.40	821	3.07
Aug. 21	129.60	390	1.92	147.20	1,486	5.56
Aug. 28	109.60	733	3.60	100.80	1,285	4.81
Sept. 4	145.60	1,140	5.60	89.60	1,031	3.93
Sept. 11	136.80	1,652	8.12	99.30	1,410	5.28
Sept. 18	100.00	1,112	5.47	108.80	1,726	6.46
Sept. 25	84.00	727	3.57	118.40	1,831	6.85
Oct. 2	82.40	475	2.34	912.00	1,624	6.08
Oct. 9	119.20	1,399	6.88	124.00	2,208	8.27
Oct. 16	132.00	3,430	16.83	115.20	2,780	10.42
Oct. 23	124.00	2,655	13.05	92.00	2,738	10.25
Oct. 30	111.20	3,195	15.71	75.20	2,107	7.89
Nov. 6	93.60	..........*	....	93.60	944	3.53
Nov. 13	..........*	....	....	....	1,485	5.56
Nov. 20	....	....	....	....	1,164	4.36
	1928			1929		
Season's total	..........*	28,223†	100.00†	....	23,110†	100.00†
Aug. 7	65.60	515	1.82	216.00	573	2.48
Aug. 14	100.80	739	2.62	193.60	658	2.85
Aug. 21	114.40	1,151	4.08	146.40	924	4.00
Aug. 28	101.60	1,560	5.53	128.00	925	4.00
Sept. 4	94.40	1,326	4.70	98.40	957	4.14
Sept. 11	90.40	1,449	5.13	115.20	1,334	5.77
Sept. 18	102.40	1,444	5.12	127.20	1,313	5.68
Sept. 25	100.80	1,619	5.74	116.80	1,689	7.31
Oct. 2	100.00	1,763	6.25	121.60	1,718	7.44
Oct. 9	100.80	1,972	6.99	107.20	1,624	7.03
Oct. 16	97.60	2,250	7.97	91.20	1,618	7.00
Oct. 23	105.60	2,459	8.71	104.00	1,435	8.37
Oct. 30	90.40	2,479	8.78	119.20	2,122	9.18
Nov. 6	23.20	1,453	5.15	114.40	2,020	8.74
Nov. 13	....	749	2.65	110.40	1,572	6.80
Nov. 20	....	1,128	4.00	139.20	874	3.78

* Dashes indicate no data available or insufficient data.
† Includes sales prior to August 1 and after November 20.

Sources of data:

Col. 1: Simple or unweighted weekly average price for lugs and crates of Cornichon, Emperor, Malaga, Thompson Seedless, and Tokay varieties multiplied by 80 to convert to approximate price per ton. Data from reference 8, *p. 23–46*.

Cols. 4, 7, 10: True or weighted average prices for lugs and crates of varieties listed above and Red Malaga in addition, multiplied by 80 to convert to price per ton. Prices for 1927 from reference 2, *p. 58–79;* for 1928 from reference 9, *p. 47–94;* and for 1929 from reference 7.

Col. 2: Estimates for Emperor, Malaga, Thompson Seedless, and Tokay varieties based upon inspections by the Federal-State Inspection Service from reference 8, *p. 75, 76*.

Cols. 5, 8, 11: Shipments of Emperor, Malaga, Red Malaga, Black Prince, Rose of Peru, Thompson Seedless, and Tokay varieties. Data for 1927 from reference 2, *p. 80–85;* for 1928 and 1929 from references 3 and 4.

It will have been observed that factors other than that of shipments have not been emphasized. While such other factors as supplies of eastern grapes, and seasonal movement and quality of all table grapes play a part in determining the season's average price of California table grapes, the greatest influence by far is that of total carlot *shipments.*

A number of factors not discussed in this article that might logically be expected to influence table-grape prices were studied but found to have had an effect too small to be apparent or else an effect obscured by other factors. The more important of these other factors were (1) United States production of certain other fruits, both individually and in combination with one another, (2) various indexes, such as those of factory payrolls, employment, and the price of foods, (3) the proportion of table-grape shipments consisting of different varieties, and (4) track holdings.

BLACK-JUICE GRAPES

As was true of table grapes, California shipments were found to have been the chief determinant of black-juice grape prices[11] in eastern markets. Moreover, the demand for black-juice grapes was found to be about as inelastic as that of table grapes (see page 104). Figure 3 shows how close the relation has been, during a period of nine years, between total inter and intrastate shipments of California black-juice grapes and weighted average annual New York delivered-auction prices adjusted for changes in the general level of all-commodity wholesale prices. The regression curve in this figure indicates that the elasticity of demand for black-juice grapes varies from about 0.8 to 0.9 with variations in shipments from about 25,000 to 31,000 carloads.

The greater divergence of the scatter from the regression curve in figure 3 as compared with that in figure 1 indicates that changes in the volume of shipments have accounted for a smaller part of changes in the price of black-juice grapes than in the case of table grapes. The fact that black-juice prices for 1925 and 1926 were so different, although the volume of shipments was practically the same, indicates a definite difference in demand in these two years. This suggests

[11] In this analysis the weighted average price of New York delivered-auction sales of the following grapes was used: Alicante Bouschet, Carignane, Petite Sirah, Mission, Mataro and Zinfandel. These six varieties comprise, on an average, about 75 per cent of the annual shipments of black-juice grape shipments from California.

a comparison of the available facts regarding these two marketing seasons as a means of discovering variables other than volume of shipments that may have affected prices. Figure 3 shows a similar, although greater, difference in demand in 1927 compared with 1928.

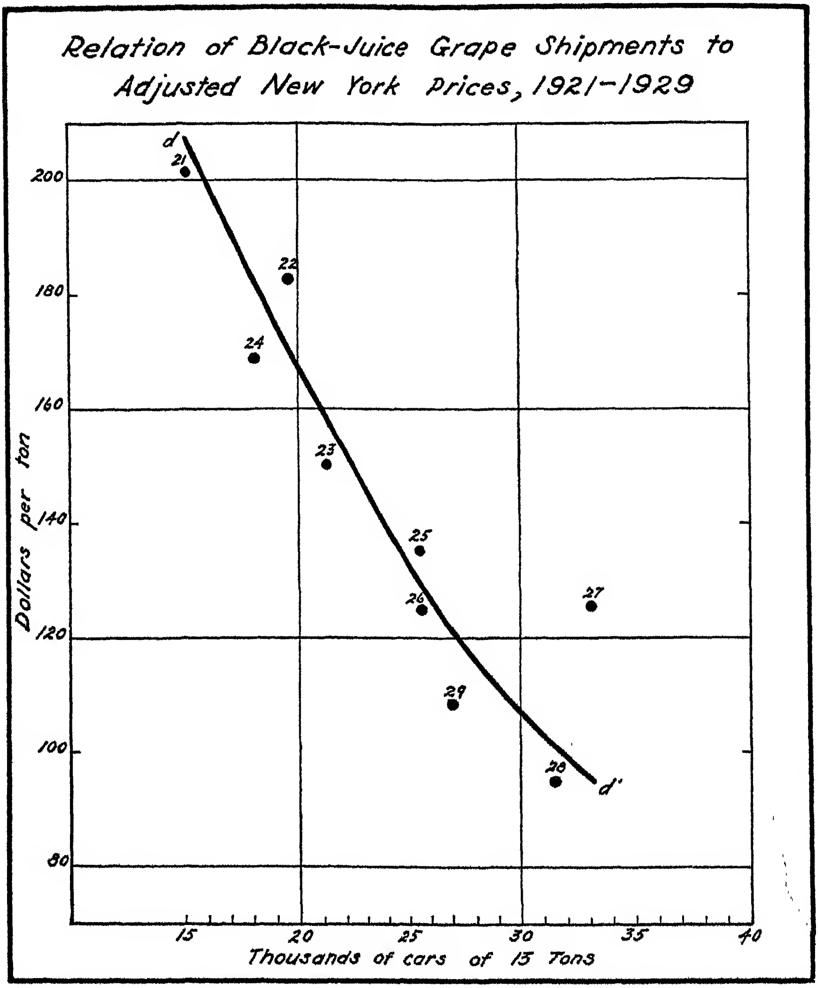

Fig. 3. Data from table 3.

By studying these four marketing seasons carefully with the aid of the general information available in trade literature, as well as the statistical record of California fresh-grape deals, a reasonable partial explanation of the causes of the differences in demand in 1925 compared with 1926 and in 1927 compared with 1928 was worked out.

This, together with other analyses indicated that the more important factors affecting black-juice prices, other than the season's volume of shipments in recent years, have apparently been (1) timing of early black-juice shipments to demand in eastern markets, (2) volume and timing of Muscat shipments, and (3) quality of black-juice stock.

TABLE 3

Annual Shipments and Actual and Adjusted New York Delivered-Auction Prices of California Black-Juice Grapes, 1921–1929

Crop year	California shipments	Price per ton	
		Unadjusted	Adjusted
	1	*2*	*3*
	carloads	*dollars*	*dollars*
1921	15,200	196.80	201.63
1922	19,600	176.80	182.80
1923	21,200	151.20	150.29
1924	18,100	165.60	168.80
1925	25,500	140.00	135.26
1926	25,600	124.80	124.80
1927	33,100	120.00	125.80
1928	31,500	92.80	95.00
1929	27,000	104.80	108.60

Sources of data:

Col. 1: Total shipments to the nearest hundred cars in carloads of approximately 13 tons net, both inter and intrastate, of all black-juice varieties—Alicante Bouschet, Zinfandel, Carignane, Petite Sirah, Mission, and Mataro are the chief ones. Data for 1928 and 1929 increased 6 per cent to allow for heavier loadings per car. Data for 1921–1926 from reference 1, *p. 46, 47;* for 1927–1929 from same sources and same methods indicated in footnote 1, table 1, page 106.

Col. 2: True or weighted average prices per lug of New York delivered-auction sales through the first or second week in November for the varieties listed above. Converted to price per net ton at the rate of 80 lugs per ton. Data from source indicated in footnote 2, table 1, page 106.

Col. 3: Prices adjusted to 1926 base by use of U. S. Bureau of Labor Statistics all-commodity index of wholesale prices for calendar years.

It is generally conceded by the best-informed marketing agencies that practically all California black-juice grapes shipped to eastern markets are used for wine-making. It is also common knowledge that the making of a good grade of wine requires a temperature conducive to proper fermentation. This study indicates that a weekly average mean temperature of 50° to 60° F is apparently the range within which the most active buying of juice grapes takes place. It will be observed in table 4 that such a temperature usually starts to prevail in New York City anywhere from the first to the third week in October, the month of greatest demand and heaviest sales of juice grapes. Such temperatures generally prevail during a large part of

this month. Judging by sales and unloads data in New York and Jersey City markets, buyers of juice stock usually allow a week to 10 days of such favorable temperature for wine-making to pass before they purchase any considerable quantities of juice grapes. Up to that time buyers appear to have usually been indifferent about purchasing juice stock.

TABLE 4

WEEKLY AVERAGE OF DAILY MEAN TEMPERATURES FOR NEW YORK CITY, IN DEGREES FAHRENHEIT, 1925–1929

Week ending	1925	1926	1927	1928	1929	Five-year average
Aug. 7	71.7	75.5	69.3	79.1	70.5	73.2
Aug. 14	74.4	76.7	70 4	75.7	73 0	74.0
Aug. 21	74.1	66.1	66.7	74.1	70.8	70.4
Aug. 28	67.9	71.3	64.4	70.0	70 3	68.8
Sept. 4	71.9	64.7	67.4	74.4	78.3	71.3
Sept. 11	71.2	66.2	69 9	65.5	71.5	68.9
Sept. 18	70.3	66.9	68.4	70 9	60.7	67.4
Sept. 25	62.4	63.9	62.7	66.1	65.7	64.2
Oct. 2	59.7	64.6	66.3	54.2	57.7	60.5
Oct. 9	55.5	54.4	68.1	60.2	53.4	58.3
Oct. 16	49.9	52.4	56.6	62.6	53.3	55.0
Oct. 23	47.9	49.4	52.8	63.9	57.0	54.2
Oct. 30	44.6	49.0	57.9	51.5	55.0	51.6
Nov. 6	44.9	48.4	52.4	47.9	49.3	48.6
Nov. 13	47.0	45.1	43.1	48.0	52.5	47 1
Nov. 20	46.7	41.3	50.8	50.7	42.4	46.4
Nov. 27	39.9	41.3	46.2	46.2	34.5	41.6

Source of data: U. S. Dept. Agr. Climatological Data.

Study of the shaded areas in figures 4[12] and 5 brings out the fact that in 1926 and 1928 sales and unloads of black-juice grapes lagged a week to two weeks behind arrival of shipments. This condition occurred because California black-juice grapes matured early and a considerable volume reached eastern markets before the proper temperature prevailed to create a strong demand for them. Apparently, because of this fact, prices opened at a lower level than they would have if heavy shipments had been delayed until demand had strengthened. The season's average price in both years was lower than the regression curve in figure 3 would lead one to expect from the season's total black-juice shipments. The tendency for prices to strengthen during the period of heavy sales in both 1926 and 1928 also suggests that demand, during the early part of the season, was not strong enough to support the heavy early arrivals.

[12] In figure 4, weekly volume of auction sales of black-juice grapes in New York City were substituted for weekly unloads because there were no available unload statistics for seasons prior to 1927.

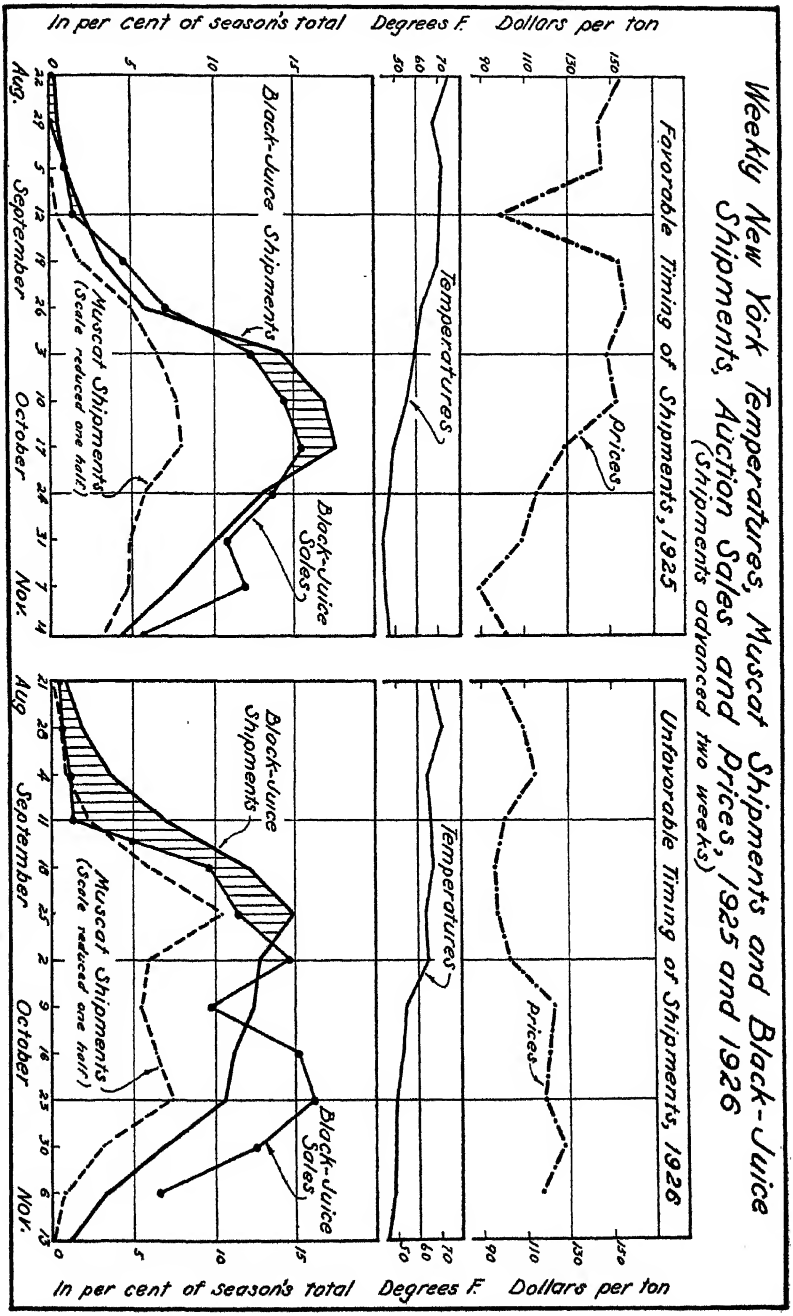

Fig. 4. The shaded areas indicate the lag between arrivals and sales in the first few weeks of each season. Data from tables 4 and 5.

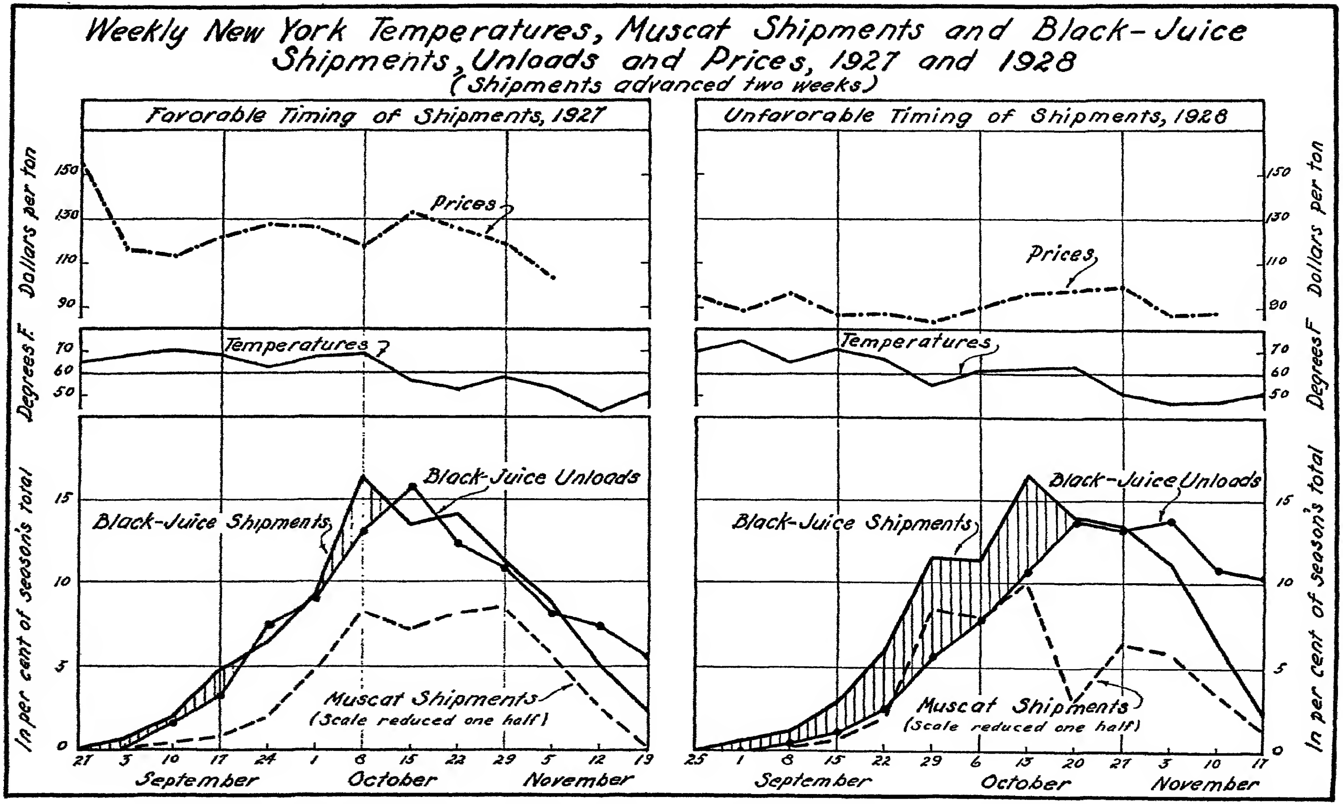

Fig. 5. The shaded areas indicate the lag between arrivals and sales in the first few weeks of each season. Data from tables 4 and 6.

On the other hand, in years like 1925 and 1927 in which heavy arrivals in eastern markets did not occur until about the time when temperature conditions were favorable for wine-making, consumers appear to have been in the market ready to move juice grapes into consumption at better prices than could be expected earlier. Shipments to eastern markets in these two years were delayed either because of late-maturing crops or because early shipments were held back by shipping organizations. In both 1925 and 1927, there was favorable timing of early shipments to demand, unloads coinciding closely with arrivals from California. Correspondingly, prices were above the average to be expected for the quantities shipped, judging from the regression line in figure 3. Prices in 1925, however, were not as well maintained as in 1927. The sharp price decline in the latter part of the 1925 season was largely due to very unfavorable weather conditions—early snow and heavy frosts. During 1927 there appears to have been an exceptional demand for juice grapes, the causes of which have not been entirely explained.

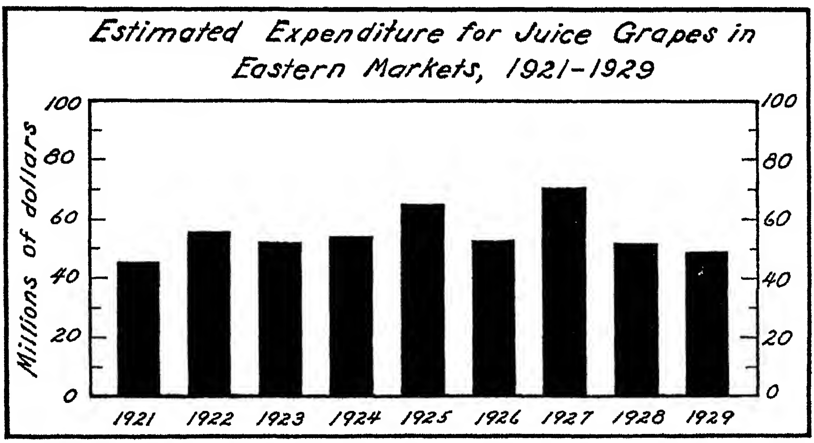

Fig. 6. Data based upon tables 3 and 7 and computed by adding the value of shipments of black-juice grapes, at their adjusted price, to the value of shipments of Muscats, at their adjusted price.

Perhaps the most important factor affecting black-juice grape prices remaining to be discussed is the volume and timing of fresh Muscat shipments. According to the trade, fresh Muscats have been extensively used in the East for blending with black-juice varieties, partly because they have been low priced and partly because many consider that they improve the color and taste of wine. Information from those acquainted with wine-making in the East indicates that

no set proportion of black and white juice is used in making a blend. When Muscat supplies are plentiful owing to heavy shipments early in the season, and their price more attractive to buyers than that of black-juice grapes, the proportion of Muscats used in blending is presumably increased.

The hypothesis that Muscats are used to hold down the aggregate cost of the wine made seems to be supported to some extent by figure 6, which gives the total outlay, based on New York auction prices, for all juice stock by years. With the exception of 1925 and 1927, the total outlay per season has remained fairly uniform, although a slight downward movement may be occurring. As black-juice grape prices have declined greatly since 1925 with increased shipments, apparently more of them have been substituted for Muscats.

Figures 4 and 5 enable one to see the relation between weekly Muscat shipments and black-juice prices, shipments, and unloads in four recent years. In 1925 and 1927, years when prices of black-juice grapes were most favorable, heavy Muscat shipments did not arrive in eastern markets before black-juice grapes were moving readily into consumption. Because shipments of Muscats were favorably timed to the demand for black-juice grapes they apparently did not tend to depress black-juice prices unduly. During the 1926 season, although black-juice shipments were not properly timed to demand, Muscat shipments arrived in the East after the demand for black-juice grapes had developed. Black-juice prices, therefore, were probably prevented from falling lower than they did. The season of 1928 illustrates the tendency for substantial arrivals of Muscats before the demand for wine-making had become well developed to add to the unfavorable conditions created by early shipments of black-juice grapes. The bulk of Muscat shipments in 1928 arrived in eastern markets before black-juice grapes were moving most freely into consumption, and thereby apparently depressed the price of both classes of grapes.

Quality of black-juice grapes has obviously had some influence upon their prices. In 1926 the good quality of black-juice grapes plus late Muscat shipments aided materially in preventing prices from remaining on a low level for the entire season. Since juice grapes are not used for direct table consumption, however, external appearance probably affects their prices less than it does table-grape prices.

TABLE 5

WEEKLY PRICES, AUCTION SALES, AND SHIPMENTS OF BLACK-JUICE GRAPES, AND SHIPMENTS OF MUSCATS, 1925 AND 1926

Weeks ending (as of 1926)	Price, dollars per ton	Sales in 11 delivered-auction markets: Number of packages	Sales in 11 delivered-auction markets: Per cent of season's total	Black-juice shipments: Carloads	Black-juice shipments: Per cent of season's total	Muscat shipments: Carloads	Muscat shipments: Per cent of season's total
	1	2	3	4	5	6	7
1925							
Season's total*	.... †	**6,293,667**	**100.00**	**28,218**	**100.00**	**19,573**	**100.00**
Aug. 7	....	....	0	81	0.27	....	....
Aug. 14	168.00	1,000	0.02	87	0.35	....	....
Aug. 21	155.20	13,656	0 20	261	0.92	19	0.10
Aug. 28	144.00	33,192	0.53	596	2.11	223	1.13
Sept. 4	145.60	56,792	0 90	970	3.43	733	3.74
Sept. 11	98 40	88,968	1.41	1,637	5.80	1,867	9.53
Sept. 18	153.60	288,019	4 58	4,066	14.40	2,611	13.33
Sept. 25	156.80	450,539	7.16	4,786	16.96	3,040	15.53
Oct. 2	148.80	786,519	12.50	5,057	17.92	3,157	16.12
Oct. 9	152.80	911,202	14.48	3,716	13,16	2,357	12.04
Oct. 16	129.60	974,648	15 49	2,825	10.01	1,943	9.92
Oct. 23	115.20	872,133	13.86	2,135	7.56	1,926	9.84
Oct. 30	108.80	690,100	10.96	1,373	4.86	1,334	6.81
Nov. 6	88.80	760,231	12.08	565	2.00	363	1.85
Nov. 13	101.60	366,668	5.83	63	0.21	*	*
1926							
Season's total*	....	**5,897,241**	**100.00**	**27,882***	**100.00***	**11,198**	**100.00**
Aug. 7	*	3,084	0.05	281	1.01	5	0.08
Aug. 14	91.20	15,280	0.26	530	1.90	138	1.23
Aug. 21	98.40	32,799	0.56	1,026	3.68	216	1.92
Aug. 28	109.60	36,296	0.62	1,936	6.95	505	4.50
Sept. 4	114.40	63,195	1.07	3,445	12.37	1,345	12.01
Sept. 11	100.80	78,808	1.34	4,178	14.98	2,368	21.14
Sept. 18	96.00	577,496	9.79	3,570	12.81	1,319	11.77
Sept. 25	96.80	676,241	11.47	3,506	12.57	1,251	11.17
Oct. 2	102.40	868,098	14.72	3,173	11.39	1,404	12.53
Oct. 9	123.20	576,287	9.77	2,988	10.73	1,662	14.84
Oct. 16	121.60	896,798	15.21	1,896	6.82	709	6.33
Oct. 23	118.40	952,092	16.14	912	3.28	163	1.45
Oct. 30	127.20	743,136	12.60	320	1.15	113	1.01
Nov. 6	117.60	377,631	6.40	....	....	....	....

* Data for the period August 1 through November 13 are identical with the season's totals except in the case of black-juice shipments for 1926, which amounted to 27,781 cars for that period compared with the season's total of 27,882.

† Dashes indicate no data available or insufficient data.

Sources of data:

Col. 1: Simple or unweighted weekly average price for lugs of the chief black-juice varieties—Alicante Bouschet, Carignane, Zinfandel, Petite Sirah, Mission and Mataro—multiplied by 80 to convert to approximate price per ton. Data from reference 8, *p. 23–46*.

Col. 2: Weekly totals of sales of the varieties listed above on the eleven delivered-auction markets listed in footnote 6, page 103. Data from reference 8, *p. 23–46*.

Cols. 4 and 6: Include a few hundred cars of white-wine varieties in both 1925 and 1926. Data for 1925 from reference 1, *p. 71* and for 1926 from reference 8, *p. 75–76*.

TABLE 6

WEEKLY SHIPMENTS OF BLACK-JUICE GRAPES AND UNLOADS AND PRICES IN NEW YORK AND WEEKLY MUSCAT SHIPMENTS, 1927–1929

Weeks ending (as of 1927)	Price, dollars per ton	Unloads of black-juice grapes		Interstate shipments			
				Black-juice		Muscat	
		Carloads	Per cent of season's total	Carloads	Per cent of season's total	Carloads	Per cent of season's total
	1927						
1	2	3	4	5	6	7	8
Season's total	........*	**8,069**	**100.00**	**29,140†**	**100.00†**	**15,216**	**100.00**
Aug. 13	....	....	....	28	0.09	....	....
Aug. 20	....	....	....	261	0.89	16	0.11
Aug. 27	155.20	....	....	748	2.56	149	0.97
Sept. 3	116.80	18	0.22	1,302	4.46	272	1.78
Sept. 10	113.60	131	1.62	1,909	6.55	640	4.21
Sept. 17	121.60	253	3.13	3,484	12.00	1,434	9.43
Sept. 24	127.20	612	7.58	4,752	16.30	2,628	17.28
Oct. 1	126.40	730	9.04	3,933	13.50	2,235	14.69
Oct. 8	118.40	1,058	13.11	4,282	14.70	2,533	16.65
Oct. 15	133.60	1,279	15.85	3,317	11.40	2,584	16.98
Oct. 22	126.40	996	12.34	2,601	8.92	1,741	11.44
Oct. 29	118.40	827	10.24	1,493	5.12	747	4.91
Nov. 5	104.80	662	8.20	691	2.37	143	0.93
Nov. 12	....	618	7.65	245	0.84	83	0.55
Nov. 19	....	456	5.65	78	0.27	5	0.03
	1928						
	9	10	11	12	13	14	15
Season's total	....	**5,664**	**100.00**	**26,048†**	**100.00†**	**14,170**	**100.00**
Aug. 13	79.20	2	0.04	37	0.14	....	....
Aug. 20	97.60	3	0.05	197	0.76	14	0.10
Aug. 27	94.40	11	0.19	339	1.30	56	0.39
Sept. 3	88.80	22	0.38	775	2.97	201	1.42
Sept. 10	96.00	27	0.47	1,586	6.08	596	4.21
Sept. 17	86.40	62	1.09	3,031	11.63	2,441	17.23
Sept. 24	87.20	143	2.52	3,023	11.60	2,238	15.79
Oct. 1	84.00	315	5.56	4,333	16.63	2,872	20.27
Oct. 8	90.40	440	7.76	3,650	14.01	837	5.91
Oct. 15	96.80	668	11.79	3,541	13.59	1,832	12.93
Oct. 22	98.40	780	13.78	2,972	11.40	1,667	11.77
Oct. 29	99.20	757	13.37	1,729	6.63	1,009	7.12
Nov. 15	87.20	789	13.94	642	2.46	347	2.44
Nov. 12	88.00	621	10.97	115	0.44	47	0.33
Nov. 19	....	573	10.11	26	0.09	9	0.06
	1929						
	16	17	18	19	20	21	22
Season's total	....	**7,632**	**100.00**	**24,294†**	**100.00†**	**8,855**	**100.00**
Aug. 13	....	....	....	....	....	....	....
Aug. 20	....	....	....	7	0.02	....	....
Aug. 27	135.20	2	0.02	149	0.61	4	0.04
Sept. 3	132.80	17	0.22	412	1.69	19	0.21
Sept. 10	99.20	20	0.26	837	3.44	27	0.30
Sept. 17	103.20	54	0.71	1,625	6.68	264	2.98
Sept. 24	106.40	104	1.36	2,548	10.49	829	9.36
Oct. 1	104.80	254	3.32	4,094	16.86	2,295	25.92
Oct. 8	90.40	421	5.52	3,724	15.33	1,592	17.98
Oct. 15	94.40	614	8.05	2,666	10.98	1,390	15.70
Oct. 22	103.20	1,139	14.93	3,272	13.46	1,207	13.64
Oct. 29	113.60	1,099	14.39	2,747	11.31	761	8.60
Nov. 5	107.20	1,094	14.34	1,678	6.91	316	3.57
Nov. 12	102.40	1,064	13.95	455	1.87	107	1.21
Nov. 19	122.40	859	11.25	80	0.33	43	0.47

* Dashes indicate no data available or insufficient data.

† Prior to August 7 only one car of black-juice grapes was shipped in 1927, only 21 cars in 1928, and none in 1929. In all other cases the difference between the season's total and the total from August 7 to November 19 are figures for the season after November 19.

Sources of data:

Cols. 2, 9, and 16: True or weighted average New York delivered-auction prices per lug of chief black-juice varieties—Alicante Bouschet, Zinfandel, Carignane, Petite Sirah, Mission and Mataro—multiplied by 80 to convert to price per ton. Data for 1927 from reference 2, *p. 75–79;* for 1928 from reference 9, *p. 47–98*, and for 1929 compiled from reference 7, supplemented by reference 4.

Cols. 3, 10, and 17: Unloads in New York and Jersey City compiled from daily Market News on Grapes issued by U. S. Dept. Agr. Bur. Agr. Econ. from Fresno. See reference 7, for 1929.

Cols. 5 and 7: Compiled from reference 2, *p. 20–26.*

Cols. 12, 14, 19, and 21: Compiled from references 3 and 4.

Undoubtedly, there are other factors which have influenced the price of black-juice grapes, such as strikes (e.g., the truckmen's strike in New York City in 1929), racketeering, trends in prohibition enforcement, and psychological peculiarities of purchasers in eastern juice markets. Some of these factors are unpredictable, but no doubt they have had considerable influence on black-juice grape prices at times.

MUSCATS

In pre-war days most California Muscats were dried and very few were shipped fresh to eastern markets. Since the War, however, in response to eastern demand for juice grapes and because of decreased demand for Muscat raisins, California shipments of fresh Muscats increased very rapidly. Table 7 shows that only 3,300 cars were shipped in 1921, as compared with the peak of 19,300 carloads in 1925. Since 1925, Muscat shipments from year to year have fluctuated greatly with no trend particularly evident. However the plentifulness of black-juice grapes may be sufficient to restrict Muscat shipments in the future.

Although a few fresh Muscats are sold as table stock, a very large majority are utilized for juice purposes. During the years covered in this study, Muscats alone have comprised some 80 per cent of the total shipments of California white-juice grapes, and shipments and prices of this one variety are believed to be fairly representative of white-juice grapes as a class.

Figure 7 shows that fresh Muscat shipments have apparently been the chief factor affecting eastern prices of this variety, but it also indicates that changes in demand have had an important influence on prices. The regression curve indicates that the demand for fresh Muscat grapes is very elastic. Hence the total sales value of these grapes normally is much greater for heavy shipments than for light. This is quite in contrast with the somewhat inelastic demand for table and black-juice grapes (see pages 104 and 111) and the very inelastic domestic demand for raisins.[13] The elasticity of demand indicated by the curve in figure 7 varies from about 2.0 with shipments of 8,000 to 10,000 carloads of Muscats to about 1.4 with shipments of 15,000 carloads or somewhat less. Demand is most elastic when shipments are small and prices high. The very elastic demand for fresh Muscats may be partly accounted for by the fact that a majority of eastern

[13] See accompanying paper: Shear, S. W. and R. M. Howe. Factors affecting California raisin sales and prices, 1922–1929, Hilgardia 6:78. 1931.

juice-stock buyers probably prefer black-juice grapes if the price is relatively low compared with Muscat prices, which has been the case normally in recent years with heavy shipments of black-juice grapes. In addition the regression curve in figure 7 suggests that there may be enough buyers who prefer Muscat wine or blends with black-juice so that they are willing to buy 8,000 to 12,000 carloads at moderate prices even when black-juice grapes are about the same price.

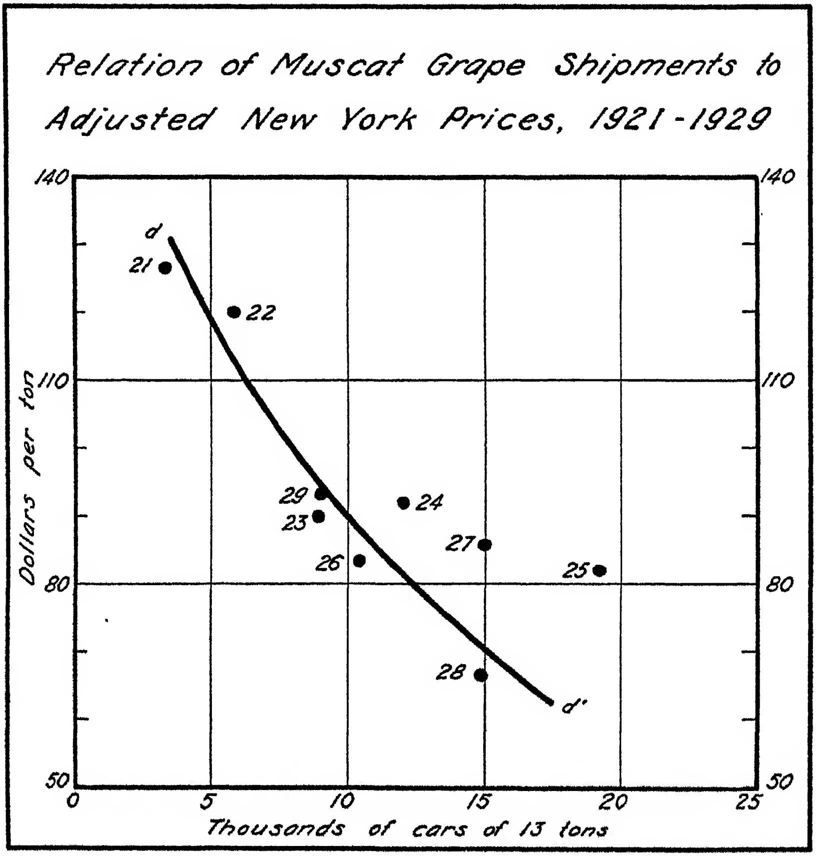

Fig. 7. Data from table 7.

Because Muscat grapes are mostly utilized for wine-making by eastern buyers, one would logically expect to find changes in demand similar to those for black-juice grapes. Comparison of figures 3 and 7 establishes this presumption as a fact. There have been very evident and significant similarities in changes in the demand for these two classes of juice grapes in recent years. Figures 3 and 7 both indicate

that there was a much greater demand for juice grapes in 1925 than in 1926. Almost identically the same quantities of black-juice grapes were sold in 1925 as in 1926 but at an average of $10 more a ton than in 1926. On the other hand fresh Muscats averaged almost the same price in 1925 as in 1926 although shipments in 1925 were nearly double those in 1926.

Prices of black-juice grapes in 1927, a year of exceptionally heavy demand for juice stock, averaged approximately $30 a ton above the prices received for 1928 shipments, which lacked only about 2,000 cars of being as large as 1927 shipments. A strikingly similar situation prevailed for fresh Muscats. Although practically the same number of carloads of fresh Muscats were shipped in both of these years, their price in 1927 averaged nearly $20 a ton above the average of 1928.

Figure 7 also brings out the fact that the demand for fresh Muscats in 1924 was noticeably better than in 1923, since substantially more Muscats were shipped in 1924 at a slightly higher price than in 1923. It is also evident that eastern demand for fresh Muscats was about the same in 1929 as in 1923 and considerably less than in 1924, 1925, and 1927. The demand for black-juice grapes was also poor in 1929. However, it may not have been much poorer than the level of demand that some well-informed men in the juice-grape marketing business fear may prevail during the next few years. Preliminary data indicate that the demand for black-juice grapes in 1930 was just about the same as in 1928 and in 1929, while the demand for fresh Muscats in 1930 appears to have been noticeably better than in 1929, perhaps because Muscat shipments were so small compared with black-juice grapes.[14]

The most important factors other than shipments that appear to account largely for the price residuals from the regression curve in figure 7 are, (1) timing of Muscat shipments to demand, (2) the quantity ratio between black-juice grapes and Muscat shipments, (3) the quality, and (4) the general price level. The discussion of the influence of similar factors on black-juice grape prices (see pages 113–118) in general applies so well to fresh Muscat shipments, that further discussion of these factors has been omitted here except as regards the relation of temperatures in eastern markets to the demand for fresh Muscats.

[14] Preliminary data indicate that about 29,200 carloads of California black-juice grapes were shipped in 1930, the average New York delivered-auction price being about $89 a ton actual or $103 adjusted. About 8,700 carloads of Muscats moved at a price of about $88 a ton actual or $102 adjusted.

Temperature appears to have greatly influenced the sale and movement into consumption of fresh Muscats in eastern markets in the same way as in the case of black-juice grapes. Muscats do not usually bring a favorable price until favorable temperatures prevail. Not until mean temperatures in New York City have remained between 50° and 60° F for a week to ten days, have a great many Muscats been sold, and when heavy shipments of Muscats have arrived in eastern markets much before favorable temperatures occur, as in 1928, prices have suffered. On the other hand, in years when Muscats have reached eastern markets after favorable temperatures had prevailed and black-juice grapes had started to move readily into consumption, their price was considerably higher.

TABLE 7

ANNUAL SHIPMENTS AND ACTUAL AND ADJUSTED NEW YORK DELIVERED-AUCTION PRICES OF CALIFORNIA MUSCAT GRAPES, 1921–1929

Crop year	California shipments	Price per ton	
		Unadjusted	Adjusted
	1	*2*	*3*
	carloads	*dollars*	*dollars*
1921	3,300	123.20	126.22
1922	5,800	116.00	119.95
1923	8,900	90.40	89.86
1924	12,000	90.40	92.15
1925	19,300	85.60	82.70
1926	10,300	83.20	83.20
1927	15,000	81.60	85.53
1928	14,900	65 00	66.53
1929	9,000	89.60	92.85

Sources of data:

Col. 1: Total shipments to the nearest hundred, both inter and intrastate, in carloads approximately 13 tons net. Data for 1928 and 1929 increased 6 per cent to allow for heavier loadings per car. Source of data indicated in footnote 1, table 1, page 106.

Col. 2: Weighted average prices for New York delivered-auction sales. Source of data indicated in footnote 2, table 1, page 106.

Col. 3: Prices adjusted to 1926 level by Bureau of Labor Statistics all-commodity index of wholesale prices for calendar years.

Most of the California Muscat vineyards were originally planted for raisin production, and before the War fresh shipments took only a small part of the crop. The percentage of the total production of Muscat grapes shipped fresh, however, rose rapidly after the War from about 5 per cent in 1921 to a peak of approximately 70 per cent in 1925. In 1928 it was 60 per cent but in other recent years it has varied from about 40 to 45 per cent.

Obviously many growers have been exercising the option of either drying their Muscats or of selling them to the fresh grape market. As a result, in recent years when prevailing prices and advances for raisins were small compared with expected returns from fresh shipments, fresh Muscat shipments have been large, as in 1927 and 1928. On the other hand, when the price of the dried product has appeared relatively more favorable, more have been dried and fewer carloads have been shipped fresh.

TABLE 8

OPENING PRICES OF FRESH AND DRIED MUSCAT GRAPES

Crop year	Fresh Muscats, opening price, dollars per ton	Muscat raisins, cents per pound
	1	*2*
1921	....*	16.7
1922	....*	10.3
1923	77	7.5
1924	67	6.3
1925	90	6.3
1926	85	7.0
1927	81	6.6
1928	59	4 4
1929	98	6.5

* Adequate data not available.

Sources of data:

Col. 1: Simple or unweighted arithmetic average of sales of lugs during the first three weeks of each season in the eleven delivered-auction markets multiplied by 80 to convert to price per ton. The eleven delivered-auction market quotations for Muscats were selected in preference to the New York delivered-auction quotations in order to obtain an earlier average opening price. Data from reference 12, *p. 26;* reference 2, *p. 68;* and reference 10, *p. 42.*

Col. 2: Simple or unweighted average of the July, August, September, and October quotations for 25-pound Sun-Maid Puffed Bakery type. Compiled from data made available by the Sun-Maid Association.

Figure 8 shows graphically how this tendency has worked out in the past. The upper scatter depicts the relation between the opening price of raisins and the opening price of fresh Muscats in the eleven eastern delivered-auction markets. There has been a tendency, as the scatter indicates, for the prices prevailing for the two different kinds of uses to strike at some equality. However, when the residuals from the curve in the upper figure were plotted against fresh-Muscat shipments, it was found that in years in which the opening price for raisins was low with respect to the prevailing prices for fresh Muscats in eastern markets, more Muscats were shipped fresh, and vice versa.

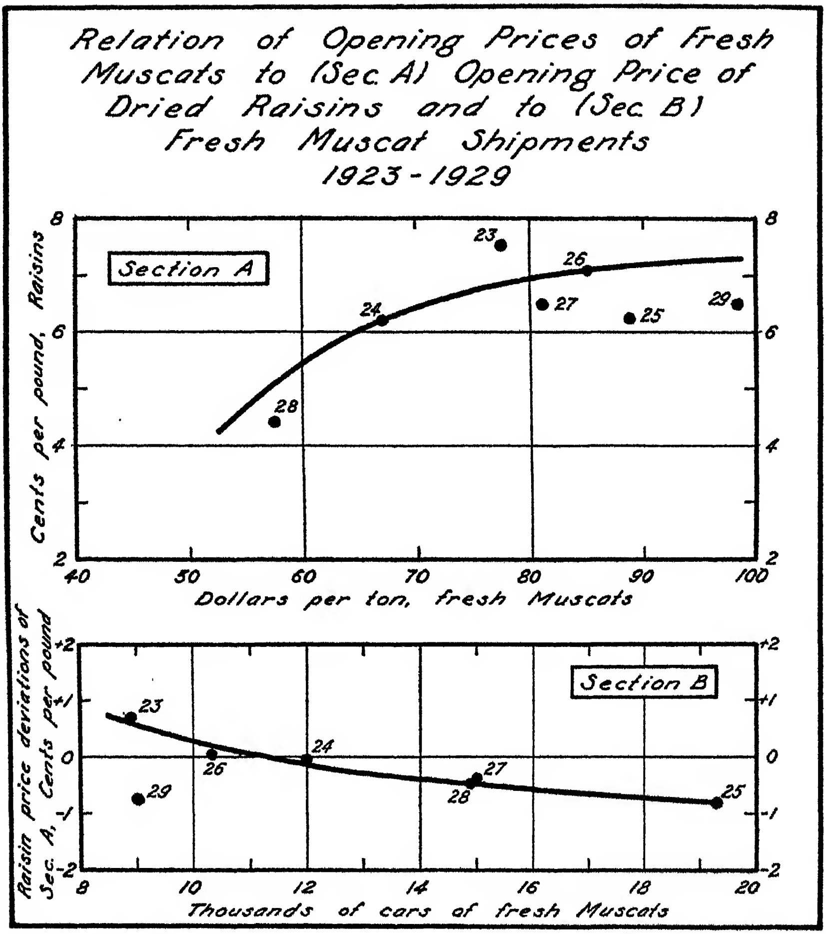

Fig. 8. Data from tables 7 and 8.

Although the 1929 season appears to be an exception to this explanation in reality it is not, for, from the second week in September to the second week in October many Muscats were dried instead of shipped fresh because of the promise unfulfilled, of a bonus of 1 cent a pound on Muscat raisins offered by the Grape Stabilization Board[15] to reduce fresh Muscat shipments and thereby lessen competition with black-juice grapes in eastern markets. Naturally, more Muscats were dried and fewer shipped fresh than would otherwise have been the case. Only about 9,000 cars of fresh Muscats were shipped as compared with 15,000 in 1928, and prices secured in eastern markets were relatively higher than those received for the Muscats dried. Only about 45 per cent of California Muscat grape production was shipped fresh in 1929 compared with over 60 per cent in 1928.

SUMMARY

Similar, somewhat related, but slightly different factors account for changes in the annual average prices of each class of California grapes in eastern markets during the years 1921 through 1929. The chief factor determining the annual average adjusted price of each class of grapes—table, black-juice, and white-juice—has been the season's total shipments. Shipments for the season have been much more influential in determining the season's average price in the case of table grapes, however, than in the cases of black-juice grapes and of Muscats. This seems to be primarily because shipments of table grapes have been better timed to meet the current needs of eastern markets than have those of juice grapes.

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[15] In the third week of August, 1929, the Federal Farm Board announced that it was taking part in a financing program whereby credit from the Farm Board and California banks amounting to 9 million dollars would be made available to Sun-Maid to be used for advances on the basis of 3 cents a pound for both Thompsons and Muscats to raisin growers who belonged to or pooled with Sun-Maid. In the second week of September, 1929, the Grape Stabilization Corporation announced that it would provide an additional advance of 1 cent a pound on Muscat raisins to this same group of growers. This one-cent bonus was designed to increase the quantity of Muscats dried and thereby decrease fresh Muscat shipments, thus reducing competition with black-juice grapes which have no alternative use other than as juice grapes. Presumably it was to be secured from assessments upon shipments of black-juice grapes, the class of grapes to be benefited most.

After getting many Muscat growers to dry their grapes by offering them this extra cent a pound, in the second week of October, 1929, the Grape Stabilization Corporation announced that it did not have the money to meet its agreement and hence the bonus could not be paid. (See California Fruit News 80 (2146) *p. 3* and *6*, Aug. 24, 1929; 80 (2149) *p. 3*, Sept. 14, 1929; and 80, (2153) *p. 3* and *6*, October 12, 1929).

The demand for black-juice grapes and for table grapes at the quantities marketed in recent years has been somewhat inelastic, although not nearly so inelastic as that for raisins.[16] The demand for fresh-Muscat grapes has been rather elastic.

The demand for table grapes does not change so much during the season as the demand for juice grapes, which apparently depends to a large extent upon temperatures favorable to the making of good wine. The bulk of sales of juice grapes, both black and white, in eastern markets has generally occurred during the month of October after mean temperatures of 50° to 60° F have prevailed for a week or ten days. In years in which black-juice grapes and Muscats arrived in eastern markets in considerable volume a week or two before proper temperatures prevailed and active buying began, prices started at a lower level and averaged less for the whole season than in years with comparable total shipments in which substantial arrivals were delayed until active demand for juice grapes prevailed.

The uses for the bulk of table and juice varieties are so dissimilar that shipments of juice grapes were found to have no measurable effect on table-grape prices, and apparently table grapes have little influence on juice-grape prices, except insofar as any considerable tonnage of a table variety like Malagas are diverted into juice-grape channels. However, shipments of eastern *labrusca* grapes, the majority of which are probably used for table purposes, were found to affect the price of California table grapes to a minor extent.

Though there can be no doubt that quality has a substantial effect upon the eastern price of California grapes, lack of appropriate data have unfortunately precluded statistical measurement of its influence.

Because Muscat grapes are mostly utilized for wine-making by eastern buyers, there have been very evident and significant similarities in changes in the demand for black-juice grapes in recent years. Fresh Muscats have been extensively used in the East for blending with black-juice varieties and therefore these two classes of grapes appear to have both a complementary and a competitive relation to one another. Apparently there is a tendency to increase the proportion of whichever class of juice grapes is relatively the cheaper. Owing to the large increase in black-juice shipments, they have been relatively cheaper than Muscats, and apparently, therefore, a larger proportion of blends have been made from black-juice grapes in recent years. Partly as a result of variations in blending due to changes in

[16] See accompanying paper: Shear, S. W., and R. M. Howe. Factors affecting California raisin sales and prices, 1922–1929. Hilgardia 6:73–100. 1931.

relative prices, consumer annual outlays for juice stock have remained fairly uniform during the past nine years.

Fresh Muscat shipments, and hence prices, naturally are influenced by prevailing prices and expected advances for Muscat raisins at shipping time. As a result, in recent years in which prevailing prices and expected advances for raisins have been small compared with expected or prevailing returns from fresh shipments, the fresh-Muscat movement has usually been relatively large. On the other hand, when the raisin-price outlook appeared favorable, fresh-Muscat shipments have normally been curtailed and more dried for raisins.

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THE PROPAGATION OF CITRUS BY CUTTINGS[1]

F. F. HALMA[2]

The commercial method of propagating citrus in the United States consists of budding the desired variety onto a seedling rootstock. The principal commercial scion varieties in California are Eureka and Lisbon lemon (*Citrus limonia* Osbeck),[3] Valencia and Washington Navel orange (*Citrus sinensis* Osbeck), and Marsh grapefruit (*Citrus grandis* Osbeck). The standard rootstocks are seedlings of sweet orange (*Citrus sinensis* Osbeck), sour orange (*Citrus aurantium* Linn.), grapefruit (*Citrus grandis* Osbeck) and to a limited extent rough lemon (*Citrus limonia* Osbeck). Since a budded citrus tree is a combination of either two species or two varieties of the same species, a study of the effect of the rootstock variety, or the effect of the presence of a bud union, must necessarily include a comparison of budded trees with unbudded trees, that is, with trees propagated by cuttings.

The writer's investigation of cutting propagation has been undertaken primarily because of its bearing on the problem relating to stocks for citrus budding. However, this method may be useful also in commercial propagation of citrus, or in the production of plants for experimental physiological and pathological study.

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[3] The taxonomic nomenclature is that of Swingle.(11)

It has long been known that the citron and the lemon may be propagated readily by cuttings, but writers on the subject apparently doubt the possibility of propagating the sweet orange and the grapefruit by this method. Swingle and his associates[12] have used a method similar to that employed by the writer but found that the sweet orange was difficult to propagate from ordinary cuttings, no matter how carefully they are handled. Coit[1] is of the opinion that orange cuttings are so difficult to grow that this method of propagation is altogether impracticable. Hume[10] expresses a similar view, stating that cuttings of the grapefruit and orange are more difficult to root than the lemon, and though it may be accomplished, this method for these trees has little to recommend it and is not commercially practicable.

Preliminary experiments at the University of California Citrus Experiment Station conducted by the writer in 1926[5] showed that, although the sweet orange and grapefruit root less readily than the lemon, yet a commercially satisfactory percentage of rooted plants can be obtained if the cuttings are taken from healthy vigorous trees, and the leaves on the cuttings are left intact.

In view of the fact that this investigation has been discontinued it was thought advisable to publish the results obtained from 1926 to 1930. This paper deals with the propagation of twig and root cuttings and with grafted twigs handled like cuttings.

EXPERIMENTAL METHODS AND RESULTS WITH CUTTINGS

Cuttings 10 to 15 cm long, possessing five to six nodes, are made from the mature terminal growth. With oranges and grapefruit, experience has shown that it is important to take the material from healthy, vigorous trees and that the leaves of the cuttings be healthy, mature, and free from injury. The lower two or three leaves are removed, thus leaving three or four leaves on the cuttings (fig. 1). Retention of the leaves is essential for satisfactory results; reducing the leaf area by cutting off the terminal half of the leaves has been found to retard and to reduce the amount of roots produced by the cutting.[4] Table 1 shows that while lemon leaves without stem (fig. 2) rooted satisfactorily, the seven-node stem cuttings without leaves failed to produce a measurable amount of roots. If an abundance of material is available, longer cuttings with correspondingly greater number of leaves may therefore be used with advantage.

[4] The relation of leaf area to root production will be discussed later.

TABLE 1

RELATION OF QUANTITY OF LEAVES TO QUANTITY OF ROOTS PRODUCED; EUREKA LEMON CUTTINGS GROWN FOR 55 DAYS (NOV. 29, 1929 TO JAN. 23, 1930)

Type of cutting	Number of leaves	Number of rooted cuttings	Fresh weight, per cutting: Leaves	Fresh weight, per cutting: Roots	Weight of roots per 100 grams of leaves
			grams	*grams*	*grams*
Leaf	1	29	1.56	0.24	15.38
Stem*	0†	4	....	....	....
	1	45	1.37	0 35	25.55
	2	41	2.33	0.52	22 32
	3	46	3.96	0.74	18.69
	4	46	4.78	1 02	21.34
	5	36	5.00	0.95	19.00
	6	9	7.39	1 31	17.73
	7	13	7.91	1.34	16.94

* All stem cuttings possessed seven nodes with the exception of cuttings with six and seven leaves which had nine nodes.

† Only 4 out of 27 cuttings rooted and the amount of roots produced was insignificant. The other lots rooted close to 100 per cent.

Whether the basal cut is made immediately below a bud or above is not important, but there seems to be a relation between the degree of slope of the basal cut and the number of roots which develop; the greater the slope the fewer the number of roots produced. This is shown in table 2. When a 90° cut was made and then four narrow equally-spaced grooves were made at the cut end, thus limiting root activity to four definite places, a considerable increase in number of roots occurred, although the amount of roots per unit weight of leaf was not increased.

TABLE 2

EUREKA LEMON CUTTINGS SHOWING THE EFFECT OF TYPE OF BASAL CUT ON THE NUMBER OF ROOTS PRODUCED; FORTY-FIVE CUTTINGS IN EACH LOT*

Type of cut	Number of roots: Mean	Number of roots: Standard deviation	Green weight of roots per 100 grams of green leaves	Dry weight of roots per 100 grams green leaves
			grams	*grams*
Slope of 40–50 degrees	3.3±.14	1.4±.10	16.3	1.9
Slope of 60–70 degrees	3.1±.14	1.4±.10	17.2	2.0
No slope	3.8±.17	1.7±.12	16.4	1.9
No slope, 4 grooves	4.5±.11	1.1±.08	16.0	1.8

* The total green weight of stems for each lot varied from 64 to 67 grams.

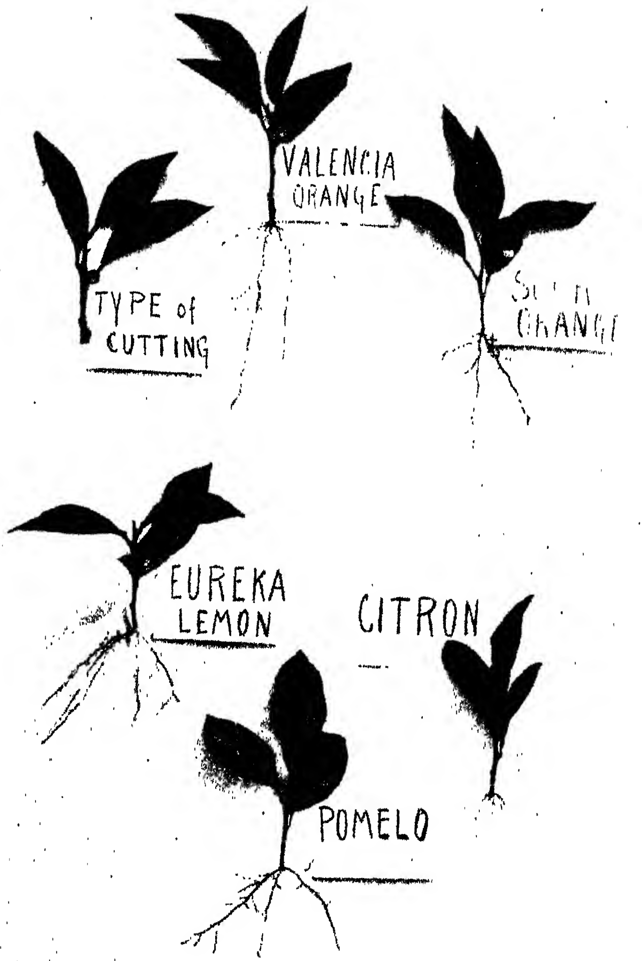

Fig. 1. Type of cutting used in propagation and several varieties of rooted cuttings 6 weeks old.

Fig. 2. Rooted Eureka lemon and Valencia orange leaf cuttings.

The cuttings are placed in sand in a sash-covered propagating frame and shaded with material such as burlap to prevent leaf burn. Until roots have developed, the leaves must be kept turgid by the maintenance of high humidity. This requires strict limitation of ventilation and frequent sprinkling with water. During cool weather, bottom heat is necessary to insure rooting of orange and grapefruit cuttings and is beneficial in the case of the lemon. Satisfactory results have been obtained by maintaining the temperature of the sand at 24° to 26° C (75° to 79° F). Cuttings can be successfully rooted in hot weather, however, when the temperature of the sand often rises to 43° C and the air is doubtless still warmer.

Some of the varieties of *Citrus* and species of genera closely related to *Citrus* which have been grown successfully from cuttings are listed in table 3. Where a sufficient number of trials were made, the average percentage of rooted cuttings obtained is noted.

TABLE 3

A List of Varieties of Citrus and Species of Genera Closely Related to Citrus Which Have Been Grown from Cuttings

Variety	Average per cent of rooted cuttings
Citrus limonia, Osbeck (lemon)	98
Citrus medica, Linn (citron)	100
Citrus aurantium Linn (sour orange)	92
Citrus sinensis, Osbeck (sweet orange)	85
Citrus grandis, Osbeck (grapefruit)	75
Citrus nobilis, Lour. var. deliciosa, Swingle (Mandarin orange)	75
Citrus nobilis, Lour. var. unshiu (Satsuma or Unshiu orange)	....
Citrus webberii	....
Citrus mitis, Blanco (Calamondin orange)	....
Citrus hystrix	....
Citranges (Hybrids between Citrus sinensis and Poncirus trifoliata)	....
Aegle marmelos, Correa	....
Atalantia citrioides, Pierre	....
Balsamocitrus dawei, Stapf	....
Balsamocitrus gabonensis, Swingle	....
Chalcas exotica, Millsp. (Orange Jessamine)	....
Citropsis schweinfurthii, Swingle and M. Kellerman	....
Citropsis gabonensis, Swingle and M. Kellerman	....
Clausena lansium, Skeels	....
Fortunella margarita, Swingle (kumquat)	....
Lavanga alota	....
Microcitrus virgata	....
Poncirus trifoliata, Raf	....
Severinia buxifolia, Ten	....

Fig. 3. Navel orange cuttings. Upper one three years old showing curled roots due to pot-bound condition at time of planting in nursery. Lower one two years old was transferred directly from the propagating frame to the nursery.

The percentage of cuttings which root may vary considerably in the same variety if the material is taken from weak trees. For example, cuttings taken from fifteen Valencia orange trees of the same age and growing in the same orchard rooted 60 per cent to 97 per cent, the weaker trees giving the lower percentages. Again Navel orange cuttings taken from eleven trees in the same orchard in 1926 averaged 87 per cent, while two years later material taken from the same trees which were then declining in vigor averaged 57 per cent.

Generally, after two or three months the cuttings will have developed fairly extensive root systems (fig. 1). Before transplanting to the nursery the plants must be hardened by gradually lowering the atmospheric humidity, which is done by admitting outside air into the frame. The plants are then lifted from the sand and planted bare-rooted in the nursery row. New growth is removed but the original leaves are left, as experience has shown that they are essential to the establishment of the transplanted cutting. Potting the plants from the cutting bed not only entails additional expense and labor but also results in curled roots, a condition which becomes worse as the trees get older (fig. 3). If the plants have well-developed root systems they can be transferred directly to the nursery even though the weather be hot and dry, provided they are watered immediately and protected from direct sunshine by shading. This is usually accomplished by the use of one or two shingles. Since young cuttings have a shallow root system, deep cultivation in the root zone must be avoided. Figure 4 shows one-year-old Valencia orange cuttings in the nursery.

The plants are left in the nursery until they are large enough for planting in the orchard. Lemon cuttings generally reach the size of saleable budded trees in two years and the sweet orange and grapefruit in two or three years (figs. 5, 6, 7).

The method just described appears to be the most practicable for the conditions and needs of the citrus industry in California. There are doubtless other, and perhaps equally successful, means of propagating citrus trees by cuttings. The lemons and citron, for example, can be successfully propagated by planting directly in the nursery larger cuttings devoid of leaves, though in this case the plants require more time to develop. For the sweet orange and grapefruit this procedure has not been found successful.

Fig. 4. Valencia orange cuttings in nursery, one year old and trained to one stem. Note the variation in height although they are progenies of one tree.

Fig. 5. Navel orange cutting in nursery, three years old, not trained to single stem. The upright growth (nearly 2 meters high) developed during the third year.

Fig. 6. Lisbon lemon cutting in nursery three and a half years old, which made a well-shaped tree without training; about 2½ meters high.

Fig. 7. Marsh grapefruit cutting in nursery three years old which made a low but shapely tree without training; over 1½ meters high.

CUTTINGS VERSUS BUDDED TREES

There are no indications in the nursery that trees grown from cuttings are inferior to budded trees, but a comparison as to their fruitfulness and longevity cannot be made until the trees reach maturity, and this comparison should be made where both kinds of trees represent the same scion strain. Assuming, however, that cuttings may be as satisfactory as budded trees it may be of interest to point out the advantages and disadvantages in the two methods as the writer sees them at this time.

Advantages of Cuttings.—1. Cuttings can be made at any time of the year, while budding is confined to the growing season.

2. Less time is required to grow a tree of suitable size for planting in the orchard. The principal reason for this lies in the fact that cuttings grow uninterruptedly in the nursery until they are set out in the orchard, whereas the growth of the root system of the budded seedling is checked when the top is cut off to force the bud into growth.

3. If a tree on its own root is killed back to the ground, a sprout from any living part will be of the same variety as the original top, but with budded trees a sprout from below the bud union will have to be budded again.

Disadvantages of Cuttings.—1. For cuttings more material has to be cut from the parent tree than for budding seedling stocks.

2. Since the lemon is very susceptible to *Pythiacystis* gummosis, cuttings of this species may be short lived in sections where this disease is important.

Doubtful Features.—1. The root system of cuttings is shallow, at least for the first two or three years. It has been observed, however, that the root system of many old budded trees on sweet orange or grapefruit rootstock is also shallow; in many cases it does not penetrate beyond 1 meter (40 inches). Nevertheless budded nursery plants have a deeper root system than cuttings, which lessens the danger that they will be blown over by wind after they have been set out in the orchard, and also facilitates balling for transplanting.

2. It is difficult at the present stage of the investigation to compare the cost of growing cuttings and budded plants because the cuttings were not grown on a commercial scale. The cost of lemon

cuttings is undoubtedly less than that of budded plants since they can be rooted in an ordinary cold frame. Orange and grapefruit cuttings, however, require bottom heat during the greater part of the year and more skillful handling than the lemon. The expense is further increased over that of lemon cuttings because the percentage of rooted orange and grapefruit is smaller.

3. It is doubtful whether greater uniformity in size of plants can be obtained with cuttings than with trees produced by budding on seedling stocks. Measurements of height of one-year-old Valencia orange and Navel cuttings and three-year-old sweet orange seedlings growing in the same nursery showed no significant difference in variability between cuttings and seedlings (table 4). Due consideration must be given however, in this case, to the difference in age of seedlings and cuttings. It is highly probable that most of the seedlings resulting from selfing of sweet orange are, as a rule, the result of apogamic (asexual) reproduction (Frost[2]). It is therefore to be expected that most of the seedlings will be identical in genetic constitution with the seed parent, and that most of the differences among them will be due to the same 'accidental' causes as with cuttings.

TABLE 4

HEIGHT IN CENTIMETERS OF SWEET ORANGE SEEDLINGS 3 YEARS OLD AND OF VALENCIA AND NAVEL ORANGE CUTTINGS 1 YEAR OLD

Orange	Number of plants	Mean	Standard deviation	Coefficient of variation
Navel orange cuttings.....	94	38.3±0.6	8.4±0 4	21.9±1 1
Valencia orange cuttings	195	42.7±0.6	11.8±0.4	27.6±1.0
Sweet orange seedlings...	90	121.7±1.9	26.4±1.3	21.7±1.1
Sweet orange seedlings...	106	138.0±2 1	31.9±1.5	23.1±1.1
Sweet orange seedlings.	91	147.8±2.3	31.7±1.6	21.4±1.1

Aside from environmental influences, the amount of variation with cuttings depends to some extent (as is shown below) on the total original leaf area of the cutting, just as the variability of the apogamic seedlings is doubtless affected by differences in size of embryo. Also any pruning given to seedlings or cuttings will obviously influence the variability in size of plant. For example, if some of the original leaves are removed from a rooted cutting the subsequent growth of the plant will be less than that of a plant whose leaves are left intact.

OTHER APPLICATIONS OF THE METHOD — TWIG GRAFTS

In rootstock investigations it is necessary to have plants representing combinations of scion and stocks of known varieties. This can be accomplished by growing cuttings of the variety which is to serve as the rootstock, and then budding them to the desired scion variety. This method requires the same length of time as the ordinary budding method.

Fig. 8. Twigs grafted together, before and after rooting.

A rapid and satisfactory method developed by the writer[5] consists of tongue-grafting together two leafy twigs representing the desired stock and scion varieties, tying the graft union with raffia,

and then treating the twig graft like a cutting (fig. 8). The graft generally unites within two weeks. Rooting depends, as with an ordinary cutting, upon the variety. Limited data suggest that the rate at which a twig graft roots is governed by the variety serving as the rootstock if the stock is provided with healthy mature leaves. For example, if the rootstock is a Eureka lemon and the scion a Valencia orange, rooting proceeds at a faster rate than in the reverse combination. However, as the plants become established in the nursery the rate of growth seems to be governed by the scion variety.

Plants propagated by this method compare favorably with budded plants, but the important advantage lies in the fact that strong plants representing various combinations can be developed within one year and plants suitable for water cultures within a few months. This method may also offer a means by which congeniality between untried citrus varieties can be tested.

THE PROPAGATION OF THE ROOTSTOCK OF MATURE TREES

Mature citrus trees of the same age often exhibit a great variation in size and yield. In orchards where soil and other conditions for growth are fairly uniform and the rootstocks are all of the same species, variation is often due to differences in scion strain. In some cases this is quite apparent, but in other cases it is necessary to grow the progenies of the scions to obtain the proof (Halma[9]). It is obvious, however, that stocks, all of one species, may be of different types (varieties or strains). Since citrus stocks are grown from seed and this seed is obtained from various sources, it is probable that stocks of one species such as sweet orange, for example, may include types differing as widely as any of the horticultural varieties like the St. Michael and the Valencia. There may also be less marked differences such as occasionally originate by bud variation within a budded variety and are commonly called strains. Such types may differ in various ways, as in general vigor, in soil and climatic adaptation, and in congeniality with particular scion varieties and even particular types of those varieties. A thorough investigation of tree variability therefore should include a study of the rootstock type. For example, if rootstock progenies were available it would be a simple matter to effect a recombination of the scion and stock strain of a given tree and thus find whether in the case of a superior tree the scion and

stock strains are especially congenial, or whether in a poor tree there is lack of congeniality. Obviously any rootstock study of mature budded trees entails vegetative propagation.

In general there are three methods by which the rootstock of mature trees may be propagated without injury to the top: (1) By forcing sprouts from below the bud union and using these sprouts for bud wood or cuttings. In the writer's experience this method has failed in every instance, whether the trunk was notched or deep cuts into the wood made with a saw. (2) By severing a root and lifting the cut end of the severed root above the soil surface. It appears that the roots of young trees respond fairly readily to this treatment, but old trees do not, with exception of rough lemon, which is of minor importance in California, at least as far as old trees are concerned. (3) Propagation by root cuttings was tried by placing root pieces in the propagation frame and in soil under shade and in the nursery. A few cuttings produced weak sprouts which died before they became mature; none rooted. Attempts to stimulate root formation by injecting into the root pieces solutions of potassium permanganate, glucose, cane sugar, thiourea, sodium nitrate, and calcium nitrate failed.

Finally a method was devised (Halma[6]) which is simple and gives satisfactory results. It consists of grafting onto a root piece, about 10–15 cm long and about 1 cm in diameter, a healthy leafy citrus twig of the type used for a cutting (fig. 9). Limited observations indicate that the lemon is a more satisfactory scion variety than either the orange or grapefruit. Satisfactory material for studying congeniality may be obtained by making the desired combinations, as for example, root pieces and scion from the same tree, root pieces from a good tree and scions from a poor tree, etc.

Either the tongue or bark-graft method may be used but, if the root-piece is of sufficient size and the bark slips, bark grafting is preferable. The union is tied with raffia and the grafted root piece is placed in the propagation bench and treated like a cutting. There is no advantage in sealing the graft union with grafting wax or paraffin. Generally within one or two weeks the scion will have united with the root piece, and if the latter does not decay rootlets appear sometimes in two weeks (fig. 9). The beneficial influence of a leafy scion upon root development can be demonstrated also with grafted and ungrafted lemon roots (fig. 10).

Fig. 9. Grafted root pieces before and after rooting.

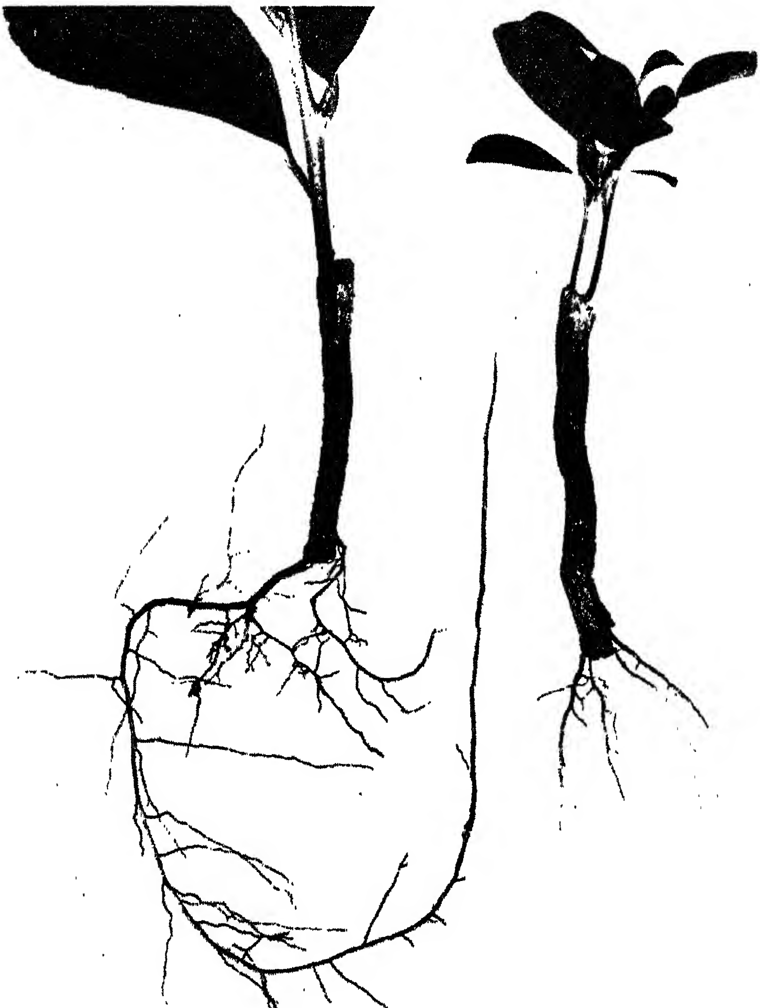

Fig. 10. Lemon root cuttings showing the quantitative difference in root development between grafted and ungrafted pieces.

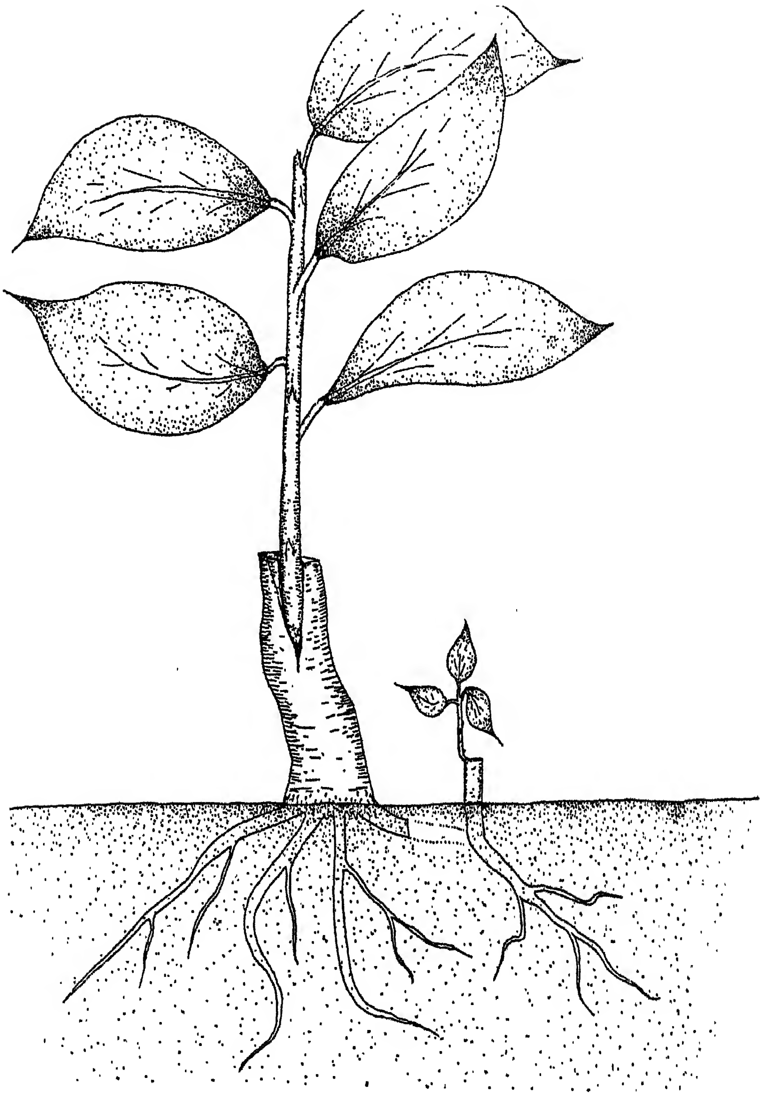

Fig. 11. Method of obtaining root sprouts from grafted root pieces.

After about three months the rooted plants are transferred to the nursery. If the plants represent combinations suitable for studying congeniality they may be set out in a permanent place in about two years. But if the plants were grown for the purpose of observing differences in rootstock strains it is, of course, necessary to induce sprout growth from the root piece by cutting off the scion. It has been found, however, that the plant must be at least two years old before this is done, or the root piece dies. Instead of cutting off the scion the safer procedure is to sever one of the young roots and raise its cut end above the ground (fig. 11); if sprouts fail to develop the mother plant is still available for further use. Limited data suggest that the best time to force sprouts from a young root or the root piece itself is during March or April.

The percentage of rooted plants obtained with grafted root pieces varies considerably more than that of twig cuttings. One lot of grafted root pieces may yield 70 per cent and another lot from the same tree only 10 per cent. This cannot be due to the scion because it remains in good condition long after the root piece has decayed. Cross sections of many apparently sound root pieces which failed to produce roots showed that the majority of the tracheae were plugged with a gummy substance, while roots which grew were free from it (figs. 12 and 13). In severe cases the gummy deposits can be seen with the unaided eye. Observations indicate that roots containing gummy deposits are most prevalent in the upper layer (about 30 cm) of soil. Furthermore, roots which have been repeatedly mutilated as a result of cultivation always show this abnormal condition even though the injury may be some distance away.

THE LEAF IN RELATION TO ROOTING

The importance of the leaf in the propagation of citrus by cuttings has already been emphasized, but it may be profitable to give experimental evidence which has a bearing on this subject. By growing leaf cuttings (fig. 2) it was found that both the area and green weight of the leaf are positively correlated with the amount of roots produced. This is also true of leafy twigs, which indicates that the stem itself plays a minor part in the rooting of the type of cuttings used (table 5). Experiments carried on with large citron cuttings without leaves and undetached lemon shoots in the orchard also showed that the amount of twig growth produced is positively correlated with the size of the cutting or undetached shoot (Halma[7]).

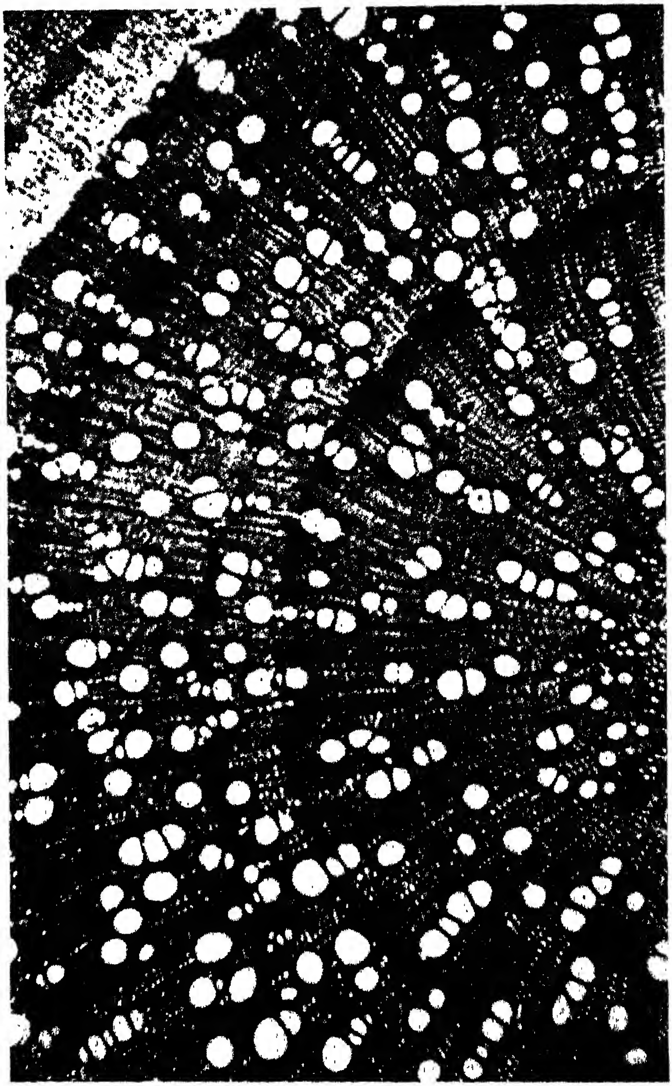

Fig. 12. Cross section of normal sweet-orange root from old tree.

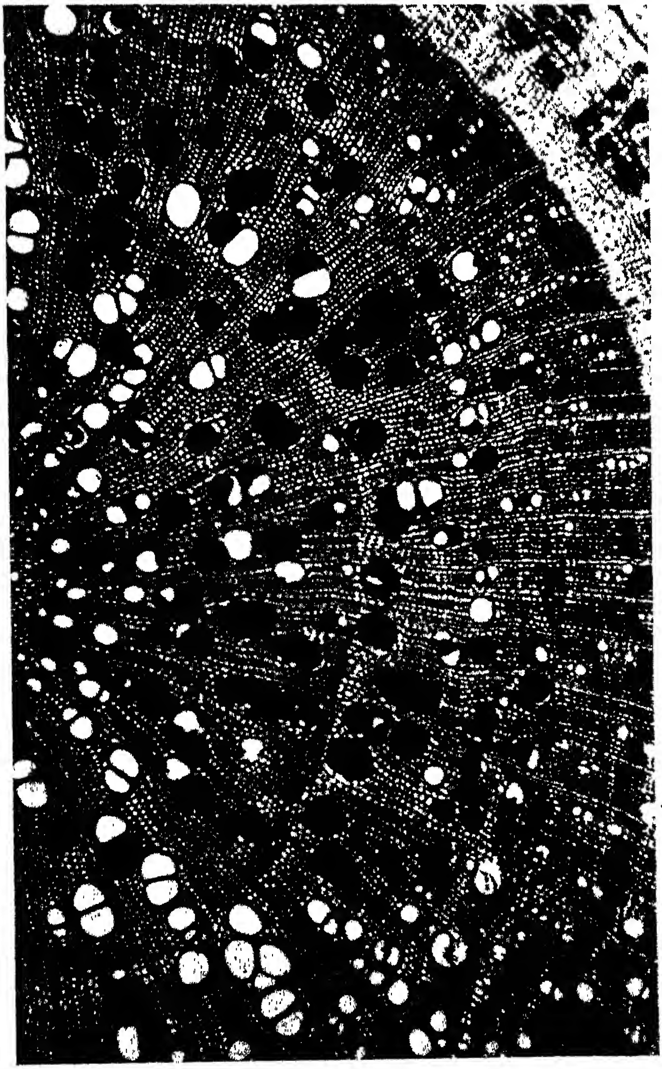

Fig. 13. Cross section of sweet-orange root from old tree, with gum deposit in trachea.

It has not been determined whether root activity is initiated by food stored in the leaf or by immediate availability of photosynthetic products made in the leaf. When a leaf is taken from the tree and placed in the propagating frame translocation must necessarily cease, and food which is not used up in respiration must accumulate until the newly formed roots can utilize it. Attempts were made to deplete the starch content of the leaves before taking them from the tree by covering them with black paper and by keeping potted plants in the dark. However, the starch did not disappear from the leaves, even after the plants had been kept in the dark room for three weeks, at which time the leaves began to drop.

TABLE 5

CORRELATION OF LEAF AREA AND LEAF WEIGHT OF SINGLE-LEAF CUTTINGS AND LEAFY STEM CUTTINGS WITH ROOT PRODUCTION

Variety	Type of cuttings	Number of cuttings	Correlation				
			Fresh weight of leaves with		Total leaf area with		
			Fresh weight of roots	Dry weight of roots	Fresh weight of roots	Dry weight of roots	Total length of roots
Eureka lemon.....	Single leaf	35	0.86±0.03	0.83±0.04	0.81±0.04	0.76±0.05	0.25±0.11
Eureka lemon......	Leafy stem	50	0.94±0.01	0.92±0.02	0.99±0.00	0.84±0.03	0.42±0.08
Valencia orange	Leafy stem	28	0.72±0.06	0.65±0.07	0.78±0.05	0.78±0.05	....

TABLE 6

QUANTITATIVE ROOT PRODUCTION OF CITRUS CUTTINGS

Variety	Number of cuttings	Period of growth	Fresh weight of roots per 100 grams of fresh weight of leaves	
			Mean	Standard deviation
		days	*grams*	*grams*
Eureka lemon*..	28	90	70.2±2.7	20.9±1.9
Valencia orange*..	22	90	28.0±1.3	8.7±0.9
Eureka lemon..	28	64	49.3±0.7	5.7±0.5
Valencia orange..	28	64	30.7±1.0	7.9±0.7
Eureka lemon..	20	77	51.0±1.1	7.3±0.8
Valencia orange..	20	77	40.5±1.7	11.3±1.2
Marsh grapefruit...	10	77	27.3 —	....
Eureka lemon*..	54	64	38.6±0.9	10.2±0.7
Navel orange*...	47	64	23.6±1.0	10.1±0.7

* Single leaf cuttings without stems (fig. 2).

TABLE 7

DEPTH OF PALISADE TISSUE EXPRESSED AS PERCENTAGE OF LEAF THICKNESS*

Variety	Sample No.	Number of leaves	Mean	Standard deviation
Chalcas exotica	1	45	31.9±0.2	2.1±0.2
Citron	1	30	30.0±0.3	2.6±0.2
Eureka lemon	1	29	28.6±0.3	2.4±0.2
	2	32	28.7±0.2	1.7±0.1
	3	30	29.3±0.2	1.6±0.1
	4	30	29.4±0.2	1.9±0.2
	5	45	28.9±0.2	2.3±0.2
	6	28	28.3±0.2	1.6±0.1
	7	25	28.8±0.2	1.7±0.2
	8	30	29.2±0.1	1.2±0.1
	9	45	28.8±0 2	1.8±0.1
	10	30	29.2±0.2	1.3±0.1
	11	30	28.2±0.2	1.8±0.2
	1–11	354	28.8±0.1	2 0±0.1
Lisbon lemon	1	30	29.0±0.3	2.3±0.2
Rough lemon	1	30	28.2±0.2	1.9±0.2
	2	30	28.9±0.2	2.0±0.2
Rusk citrange	1	39	27.6±0.2	2.0±0.2
Dancy tangerine	1	30	25.0±0.3	2.4±0.2
Sour orange	1	26	24.3±0.2	1.8±0.2
	2	30	24.8±0.3	2.1±0.2
Valencia orange	1	33	23.9±0.3	2.4±0.2
	2	30	23.3±0.2	1.3±0.1
	3	37	24.7±0.3	2.3±0.2
	4	45	23.7±0.2	1.8±0.1
	5	30	23.3±0.3	2.5±0.2
	6	25	24.3±0.4	2.7±0.3
	7	30	24.3±0.3	2.4±0.2
	8	30	23.2±0.4	3.0±0.3
	9	30	23.7±0.3	2.8±0.2
	1–9	290	23.8±0.1	3.2±0.1
Washington Navel orange	1	39	23.3±0.3	2.3±0.2
	2	30	22.8±0.2	1.6±0.1
	3	30	22.4±0.3	2.1±0.2
	4	30	22.6±0.2	1.5±0.1
	1–4	129	22.8±0.1	2.0±0.1
Marsh grapefruit	1	30	22.0±0.2	1.5±0.1
	2	30	21.6±0.2	1.7±0.2
	3	30	22.3±0.3	2.2±0.2
	4	33	21.6±0.2	1.6±0.1
	5	33	22.0±0.3	2.3±0.2
	6	30	20.7±0.2	2.0±0.2
	7	30	21.3±0.3	2.2±0.2
	1–7	216	21.6±0.1	2.1±0.1
Sampson tangelo	1	30	21.6±0.3	2.1±0.2
Owari satsuma	1	32	21 2±0.2	1.7±0.1
	2	30	20.9±0.2	1.6±0.1

*From: Halma, F. F. Quantitative differences in palisade tissue in *Citrus* leaves. Bot. Gaz. 87:319-324.

It has been pointed out that cuttings of different citrus species differ in the rate of rooting. The lemon group roots more rapidly than the sweet oranges and these in turn root sooner than the grapefruit. Table 6 shows that the amount of roots produced per unit of

fresh weight of leaf, during a given period and under the same conditions, is greater for the lemon than for the orange and grapefruit. The difference in the growth rate of the different species becomes more obvious as the cuttings develop in the nursery. For example, starting with cuttings having a similar total leaf area, the lemon, within a year, will be about twice as large as the sweet orange.

It has been shown (Halma[8]) that the depth of the palisade tissue expressed as a percentage of the thickness of the leaf is about 20 per cent greater in the lemon than in the sweet-orange leaf. The grapefruit ranks below the orange and the Satsuma mandarin exhibits the lowest value (table 7). Apparently a close relation exists between the degree of palisade development for each species and its ability to root from cuttings and also its subsequent growth rate, based on unit leaf area until the tree begins to fruit.

There are also fundamental differences between the physical and chemical constitution of the sap of Eureka lemon and that of Valencia and Navel orange (Haas and Halma[3]). The sap of normal, mature lemon leaves is less active osmotically, and contains less ash and calcium, than the sap of orange leaves. Furthermore, it has been found (Halma and Haas[4]) that the sap of lemon leaves, on exposure to direct sunshine, increases its concentration more rapidly than that of orange leaves. This increase in sap concentration is due entirely to photosynthetic products, the ash of the sap remaining the same in an exposed and an unexposed situation. While both these differences between the lemon and orange may have a bearing on the different behavior of cuttings of these species, it will be necessary to study the respiratory, photosynthetic, and translocatory processes as well before the causes for these inherent differences in behavior can be safely assigned.

SUMMARY

A method is described by which citrus trees can be grown from cuttings.

A similar method is given for rooting grafted twigs representing various combinations of scion and rootstock.

A method is described by which the rootstock type of mature trees can be propagated.

The importance of the leaf in citrus propagation by cuttings, differences in the response of citrus varieties to conditions favoring rooting, differences in leaf structure, and physical and chemical constitution of the leaf of different varieties, are discussed.

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EFFECT OF PAPER MULCHES ON SOIL TEMPERATURE, SOIL MOISTURE, AND YIELDS OF CERTAIN CROPS

ALFRED SMITH[1]

INTRODUCTION

The first large commercial use of paper as a soil covering or 'mulch' was made in 1914 by C. F. Eckart, manager of the Olaa Sugar Company in Hawaii, where it was found that when the mulch paper was applied over the row of seed cane, injury from weed growth was reduced. The use of mulch paper on sugar cane has not developed, but it has been found very useful in pineapple culture and is used at present (1931) on approximately 80 per cent of the pineapples grown in Hawaii.

Hartung[6] experimented with paper mulch as a surface covering in connection with problems dealing primarily with the use of fertilizers in the production of pineapples. The paper which he used was made of raw paper felt stock saturated with asphalt and coated with asphalt on both sides of the sheet and given a light coating of talc or soapstone which gave it a grayish brown color. He found that perforated paper did not control weeds as efficiently as nonperforated paper, and that the available soil moisture during a period of 159 days was practically the same under the perforated and nonperforated papers. Where the nonperforated mulching paper was used, he found that ammonification and nitrification of organic and ammoniacal nitrogen was more uniform, and that the soil-moisture content was

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maintained in a more favorable condition for plant growth than had hitherto been achieved in general pineapple culture. When black paper was used, the soil at a depth of 3 inches was from 3.0° to 4.5° F warmer and there was an increase of 20 to 25 per cent in crop yield.

In 1926 the Society of Manufacturers of Roofing Paper conducted tests with paper mulches on about twenty-five farms and nursery experimental stations throughout Germany, using over twenty different crops as indicators. They found that the paper maintained a better soil structure and increased the soil temperature approximately 4° to 5° F. Difficulty was experienced with plants turning yellow and wilting when tarred roofing paper was used. These harmful effects were believed to be due both to light-absorbing qualities and some chemical properties of the tarred paper. On account of these effects it was suggested that crude tar should not be used in the manufacture of mulch paper. Crop increases were obtained with the use of mulch paper on melons, cucumbers, and tomatoes. These investigators suggested that the strength of the paper and its durability need further investigation in order to determine whether the use of the paper mulch would prove profitable and practicable.

Shaw[11] at Berkeley, California, using nonperforated paper impregnated and coated on both sides with asphaltic material, found that at a depth of 3 inches below the surface "the covered plot averaged about 0.42 degree per hour warmer than the bare plot." He determined that in the surface 18 inches of soil, under the paper mulch, there was from 0.5 to 4.0 per cent more moisture than in the bare plots 6 weeks after the paper mulch had been applied. The crop yields of beans, milo maize, and potatoes indicated that under Berkeley conditions the paper covering did not favorably affect these crops.

Under field conditions in Hawaii, Stewart, Thomas, and Horner,[15] using a heavy grade of asphalt-impregnated and coated paper, determined the effect upon soil temperature, moisture, and nitrification. Temperatures were measured at a depth of 4 inches below the surface and the greatest difference between the paper-mulched and bare soil occurred during July, August, and September, and varied from 4° or 5° F during the night to frequently as high as 12° to 15° F in the afternoon. During the winter months the temperature differences were not so great. They found that in the surface foot of soil the moisture content was greater under the paper mulch than in the bare plots. The higher nitrate content found in the paper-mulched soils seemed to indicate a more rapid elaboration of the principal soil nutrients. The pineapples were 30 to 40 per cent heavier when grown with the paper mulch.

Smith[13] has reported soil temperatures obtained by use of electrical resistance thermometers under paper mulches of different colors, nonperforated and perforated. In his data obtained at Davis, California, for the warmest week in July, 1925, the temperatures at depths of 3 and 12 inches were the highest in the area covered with nonperforated black paper and the lowest under the perforated gray paper.

As a result of four seasons' work at Rosslyn, Virginia, with paper mulch, Flint[3] found, with only two exceptions, a stimulation in crop growth with the use of paper mulch. He also found perforated paper to be unsatisfactory on account of weed growth which developed through the perforations. In addition to the direct effect on yield, the nonperforated paper reduced the necessary weeding, thus adding considerably to its economic value. Flint's findings agreed with those previously reported by Smith in that the moisture conservation due to paper mulching did not extend beyond the depth of 4 inches. Flint[3] and Magruder[9] were unable to detect a greater quantity of available nitrates in the paper-mulched soil than in the unmulched soil. By the Hoffer test, Flint found that plants grown with paper mulch were higher in nitrates, while the control plants indicated either no excess nitrates or only a very slight trace. He concluded that any impervious dark paper free from toxic substances such as tar, may be as efficient in stimulating plant growth as those which are specially prepared for mulching purposes.

In a later publication[4] by Flint, it was recommended that because so little is known concerning the economic value of paper mulches, they should be tried only on a small scale and on crops having a high value. He also points out that the introduction of paper mulch into the pineapple industry of the Hawaiian Islands did not result in the reduction of the application of manures or commercial fertilizers, but it did enhance the effectiveness of fertilizers.

Edmond[2] used mulch paper on a fertilized sandy loam soil, and obtained an increase in the yield of cabbage, tomatoes, and peppers, and an increase in both yield and earliness of beans, cucumbers, and sweet corn. Of the crops mentioned, cucumbers produced the greatest increase and sweet corn the least. There was no marked influence of the paper mulch on the yield of lettuce. He stated that paper mulch should be used in an experimental way only until its practical use is definitely established.

Hall[2] experimented in South Carolina and New York in 1908 and 1909 with aluminum phosphate, which when acidified made a 'muck'

[2] William A. Hall. La Grande Estrade, Marignane, B. du Rh., France. Private correspondence.

that did not dry out. This material, worked into a fibrous body, made a transportable mulch into which he later embodied potash and nitrate. During the last nine years he has been carrying on mulching tests in southern France and has developed a mulching paper that can be worked into the soil for a fertilizer when its need for a mulch has passed. He has manufactured mats from seaweed which are reported to contain 5 to 7 per cent of potash, a fair amount of fixed nitrogen, chlorides of magnesium and sodium, and proteins and vegetable fatty matter, and are reported to be of considerable value as fertilizers.

The most general use of paper mulch at the present time is in the pineapple industry of the Hawaiian Islands, where an asphalt-impregnated, nonperforated black paper is used. Ammonium sulfate is applied as fertilizer both before the paper is laid and also around the basal leaves after the mulch is in place.

REASONS FOR THE INVESTIGATION

Heretofore most of the paper-mulch work has been in connection with its effect on crop yield, whereas in this work particular emphasis has been placed on soil temperature, soil moisture, and the effects of different types, grades, and colors of paper. Certain crops were used as indicators and the effect of the different papers and methods of laying them were carefully noted, but no attempt has yet been made to determine which of the many California crops will give the greatest response to this treatment or to go deeply into the economics of the use of paper mulch under California conditions.

LOCATION OF EXPERIMENTAL SITE

After much detailed examination an area of nearly uniform soil was selected which has a smooth surface with a slope to the southeast ranging from 5 to 10 feet per mile. The soil is the Yolo loam, an unweathered material of alluvial origin derived mainly from sedimentary rocks. The surface soil is a loam having a volume weight of 1.10. At a depth of 3 feet it is underlaid by a fine sandy loam having a volume weight of 1.13. Occasionally, at about 60 inches, sand is found. The surface water table is normally about 20 feet below the surface, and water in sufficient quantity for irrigation at approximately 120 feet. Shaw and Smith[12] found that for Yolo loam "water tables at 10 feet or more below the surface would be below

the maximum height of capillary rise and would result in no movement of water to the surface." The area was subdivided into 16 plots, 5 meters square, with a path 2 meters wide on the four sides of each plot.

INSTALLATION OF ELECTRICAL RESISTANCE THERMOMETERS

Soil temperatures were obtained by the use of electrical resistance thermometers, and an automatic Leeds and Northrup temperature recorder. Sixteen such thermometers were installed in five different plots and were standardized against standard mercury thermometers, and it is believed that all temperature data herein reported are accurate to within 0.5° F. The recorder was run continuously and adjusted so that the temperature of an individual thermometer would be registered every 15 minutes.

PAPER MULCH TRIALS IN 1925

In the first trials with paper mulch, no crop was grown because it was desired to obtain some information relative to the effect of paper mulch on soil temperature and soil moisture without interference by the shading effect of a crop. Figure 1 is a view of the experimental area showing clearly the different papers used.

Before laying the paper, the soil was worked into a granular condition and the plots were made practically level. The paper mulch was put on during the first week of May, in strips 36 inches wide with a lap of 3 inches. It was held in place by 1¼-inch redwood battens, placed over the lap and stapled with wire to the ground. The different kinds of paper mulch used are shown in table 1.

Some of the uncovered plots were cultivated 4 inches deep once or twice a month, and others were kept clean of weed growth by cutting the weeds at the surface of the soil weekly in such a way as not to disturb the soil. On May 7, after the paper mulch had been placed on the soil, moisture samples were taken by 1-foot depths. Between May 11 and 14, just after the soil sampling had been completed, there was a rainfall of 1.14 inches. Although after the rain an occasional pool of water was found on the nonperforated paper, no damage was done to any of the paper mulches other than that the flaps in the perforated papers remained open for the remainder of the season.

Soil Moisture Changes During 1925 Season.—Moisture determinations were made in all of the plots on May 7 and 22, July 6, August 10,

September 10, and October 8. The samples were dried at 212° F to constant weight, and the percentage of moisture calculated on oven-dry basis. An additional rain of 0.37 inches fell on May 18 to 20. On May 7 the moisture content of the surface foot of soil in each plot was approximately 16 per cent. Owing to the different treatments which the various plots received, the increase in moisture on May 22 was not uniform in all of the plots. The increase in moisture content of the surface foot of soil on May 22 over that of May 7 for the various plots is shown in table 1. In general, the greatest increases in moisture content occurred in the plots which had been covered with perforated

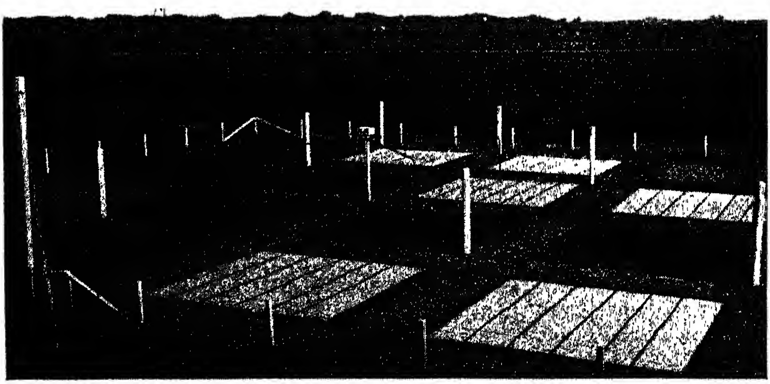

Fig. 1. Paper mulch plots in 1925 season. Various colored papers were used; no crop was grown.

paper mulch. The perforations had permitted the rain water to enter the soil, but the shading effect of the paper had retarded evaporation. Where no paper mulch had been used, a considerable portion of the rain was lost by direct evaporation to the air. Where nonperforated paper was used, moisture entered the soil through the small openings made by the staples which held down the battens.

Plot 13, mulched with Malthine Building Paper, black side up, showed a smaller increase of moisture in the surface foot than in any other paper-mulched plot. This plot contained a heavy growth of morning-glory.

Owing to rain, there was an average increase of about 2 per cent of the oven-dry weight of soil in the moisture content of the second foot in all of the plots between May 7 and May 22. There was also an average increase of about 1 per cent in the moisture content of the

third foot, while below the third foot, there was no increase in moisture during May.

Table 1 gives the percentage of the original moisture lost from the surface foot of soil between May 22 and October 8, 1925. In the areas not covered with paper the greatest loss of moisture occurred in plots 4 and 8, where the weeds had been cut at the surface of the soil

TABLE 1

MOISTURE CHANGES IN SURFACE FOOT OF SOIL DURING 1925 SEASON

Plot No.	Surface treatment*	Moisture increase, from May 7 to May 22†	Loss of original moisture May 22, to October 8‡	Moisture present October 8 based on moisture equivalent¶
		per cent	*per cent*	*per cent*
4, 8	Not cultivated, weeds cut at surface of soil	3.96	63.19	29
3, 7	Unmulched plots, cultivated once a month	3.51	52.29	43
12, 16	Unmulched plots, cultivated twice a month	2.48	51.92	46
13	Malthine Building Paper, nonperforated, black side up, white side underneath	3.60	56.18	44
11	Pabco Thermo-Gen, nonperforated, black on both sides	5.27	29.01	79
6	Pabco Thermo-Gen No. 214, perforated, large triangular perforations, black on both sides	6.41	56.61	46
5	Pabco Thermo-Gen No. 214, small round holes, black on both sides	7.51	56.17	51
1	Pabco Thermo-Gen No. 215, perforated, small triangular perforations, white side up, black side underneath	4.93	50.19	49
9	Malthine Building Paper, nonperforated, white side up, black side underneath	5.38	51.52	51
14, 15	Mulch paper plain, nonperforated, gray on both sides	4.24	45.72	54
2, 10	Moistite Thermo-Gen perforated, small triangular perforations, gray on both sides	4.32	47.71	54

* Paper on plots 1, 5, 6, 9, 11, and 13 were supplied by the Paraffine Company, Inc., and that on plots 2, 10, 14, and 15 by the Zellerbach Paper Company.

† Calculated as per cent of oven-dry weight of soil.

‡ The amount of soil moisture present on May 22 was given a value of 100 per cent.

¶ The moisture equivalent was given a value of 100 per cent. These figures are therefore ratios of moisture present to moisture equivalent.

weekly, but the soil had not been cultivated. The weeds came up through cracks in the uncultivated soil and as the season advanced, the cracks became numerous and deeper. At the time of the last sampling in October, the cracks in these plots averaged 12 inches deep and ⅛ inch wide. The least loss of moisture was in plot 11, which was covered with Pabco Thermo-Gen paper mulch, nonperforated. Although in all of these studies the surface foot of soil has been used in determining moisture changes, it has been shown by later experiments that under the conditions existing here, the conservation of moisture by paper mulches is confined to the surface 4 inches. In

general, it can be seen that where the perforated papers were used there was a greater loss of moisture, probably attributable to the circulation of air through the perforations. When the perforations were of the 'hole' type there was of course a greater opportunity for circulation of air than where the perforations were of the 'slit' type, because in the latter the small flaps would partially close the openings.

The moisture losses occurring between May 22 and October 8, in depths beyond the first foot, showed no appreciable differences in the various plots. In the second foot there was an average loss of the original moisture of about 25 per cent, in the third foot approximately 10 per cent, with lesser amounts in the fourth, fifth, and sixth foot. Where the subsoil from 3 feet to 6 feet was of a fairly uniform fine sandy loam there was slightly less moisture present on October 8 than on May 22. The changing moisture content of the soil at depths beyond the first foot is due largely to soil-moisture movement in the vapor phase, and not primarily to capillary movement. Data bearing on this point will be presented later.

Weed Growth During 1925 Season.—In plot 13, which was covered with Malthine Building Paper, difficulty was experienced with morning-glory, which continued to grow under the paper. This weed was more prevalent in this area and in the adjoining paths than elsewhere in the area under experiment. On May 17, an entire strip (36 inches wide) of paper in plot 13 had pulled loose from the battens. The weeds under the torn strip were, on the average, 2 feet apart in groups of four to five plants; they did not come through the paper, but the growth was sufficient to raise the paper, pulling it loose from the battens and wire staples. The weeds were removed and the paper replaced, the torn places being covered with additional strips of the same kind of paper.

The weeds on the other plots were largely amaranth. In plot 5, covered with Pabco Thermo-Gen No. 214, perforated and having small round holes, there were more weeds than on plot 6, covered with Pabco Thermo-Gen No. 214, perforated and having large triangular slits. Where the nonperforated papers were used, weeds came up through the holes made by the staples which held down the redwood battens. Some weeds were present in all of the plots. Those on the unmulched plots were hand-pulled regularly or destroyed by cultivation with a hoe as previously indicated. After the end of July relatively few weeds appeared on any of the plots.

Moisture-Equivalent Determinations in 1925.—Whenever soil samples were obtained they were mixed and divided into two portions, one for soil-moisture determinations and the other for moisture-

equivalent determinations. The moisture equivalent was determined by using 30-gram samples of soils,[16] the average result for the surface 3 feet being about 20 per cent, and that of the second 3 feet about 16 per cent.

Harding[5] and Veihmeyer[16] have shown that there is a fairly close relation between the moisture equivalent and the field moisture retained after an irrigation.

The percentage of moisture multiplied by one hundred and divided by the moisture equivalent is designated as percentage of moisture equivalent in table 1. It will be seen that the smallest loss of moisture was in plot 11, which retained moisture to the extent of 79 per cent of the moisture equivalent, and the greatest loss was in plots 4 and 8, which retained moisture to only 29 per cent of the moisture equivalent. In the other plots the amount of moisture present on October 8, based on the moisture equivalent, ranged from 43 per cent to 54 per cent. A comparison of the moisture equivalents of the surface foot of all the plots was made on each of the six sampling dates between May 7, 1925, and October 8, 1925, and at each date the interpretation was practically the same as given above for October 8.

Soil Temperatures in 1925.—During the period May 5, 1925, to October 1, 1925, the temperature of each of the sixteen resistance thermometers was recorded every 15 minutes day and night, and nearly 230,000 records were obtained during this period. In order to obtain a comparison of the effects of the various surface treatments on the soil temperatures, the data obtained in a 24-hour period has been segregated into day temperatures (sunrise to sunset) and night temperatures (sunset to sunrise), and the average temperatures for each day and night period during these 149 days has been calculated. In a previous paper,[14] the importance of considering the day and night temperatures separately was stressed.

Photosynthesis and transpiration in general proceed at a greater rate during sunlight than during darkness. There is less evaporation from soils at night than during the day, and according to Kincer[8] rain falling at night has less tendency to create a crust on cultivated areas than if it falls during the daytime. From records of the elongation of leaves in the date-palm, Mason's[10] results indicate this to be most rapid at night.

Soil temperatures were obtained from plots 6, 7, 10, 11, and 15, the surface treatments of which are shown in table 1. At a depth of 3 inches, during the first four weeks, the soil in plot 11 under the Pabco Thermo-Gen nonperforated paper averaged, during the day, 5.7° F warmer than in plot 7, where the soil was cultivated once a

month. The next highest day temperature during this 4-week period was in plot 6, where the surface was covered with Pabco Thermo-Gen No. 214 perforated black paper. The next in order was the cultivated plot, No. 7, followed by plot 15, covered with gray, nonperforated paper. Finally, the lowest average temperature for the first 4 weeks was in plot 10, where the surface was covered with Moistite, a gray perforated paper. During the entire period of 149 days, the black nonperforated paper produced the highest average day and night temperature at a depth of 3 inches, the gray perforated paper produced the lowest temperature, and the other papers were in the same order as during the first 4 weeks.

In order to segregate the temperature data and study the rate of heat movement to given depths in the various plots, several selected weekly periods were chosen as follows: May 5–12, June 9–16, June 23–30, July 14–21, and August 11–18. Continuous air temperatures were obtained by use of a thermograph in the Standard United States Weather Bureau shelter house at the northeast corner of the experimental area.

The period of May 5–12 was partly cloudy with the wind varying from north to southwest but with a preponderance of southwest winds. The second period, June 9–16, was generally clear with slight north winds. The third period, June 23–30, was generally clear with winds varying from north to southwest. The fourth period, July 14–21, was partly cloudy to clear with 3 days of calms and 4 days of light southwest wind. The last period, August 11–18, was generally clear with 2 days of calm, 1 of north wind, and the remainder with moderate to strong southwest winds.

The maximum of any one soil thermometer at the 3-inch depth did not occur consistently later or earlier than that of any other at the same depth. On the average the maximum temperatures at a depth of 3 inches occurred about 2 hours after the maximum air temperatures. The soil maximum temperatures, although not varying with respect to the time of occurrence, did vary in intensity, which is consistent with the results previously reported.

The average minimum temperatures at the 3-inch depth in the five plots during these 5 weeks occurred not later than 2 hours after the average minimum air temperatures.

The maximum temperatures at the 12-inch depth in the various plots occurred on the average of 7 hours and 45 minutes after the maximum air temperatures. In general, these maxima occurred within 45 minutes of each other.

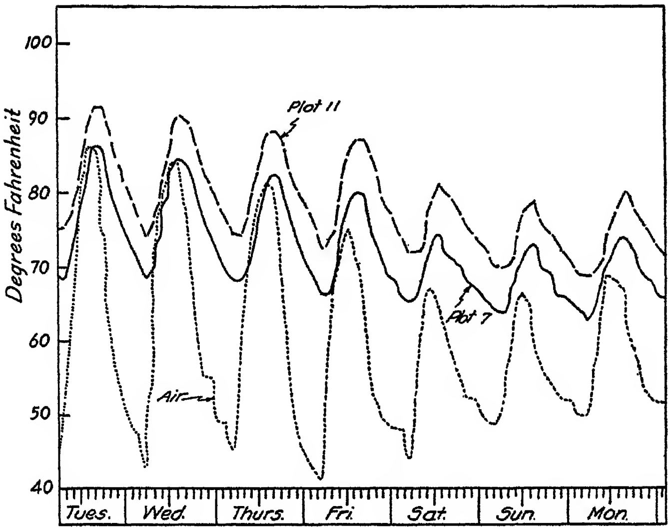

Fig. 2. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 11—Covered with Pabco Thermo-Gen nonperforated, black on both sides. Plot 7—Unmulched, cultivated once a month.

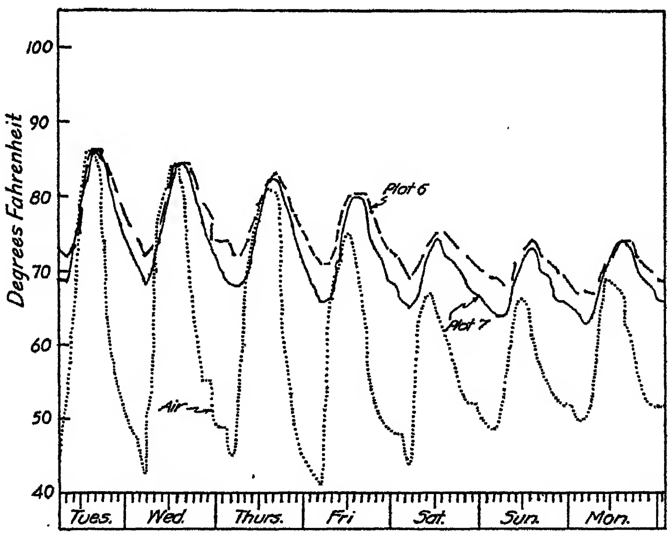

Fig. 3. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

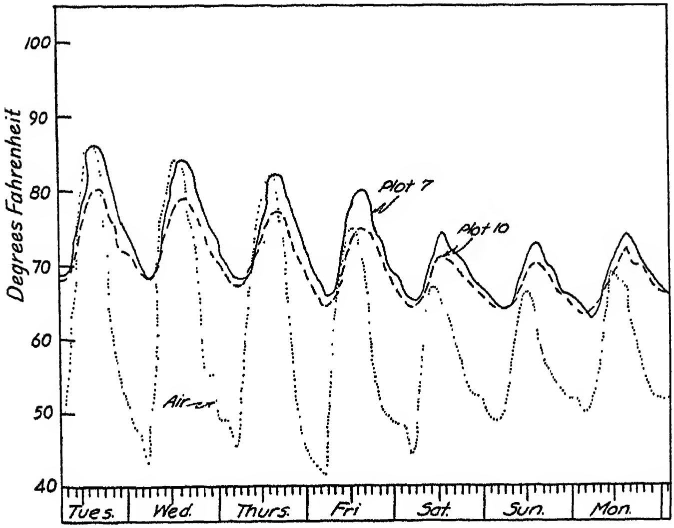

Fig. 4. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 10—Covered with Moistite Thermo-Gen perforated, small triangular slits, gray on both sides. Plot 7—Unmulched, cultivated once a month.

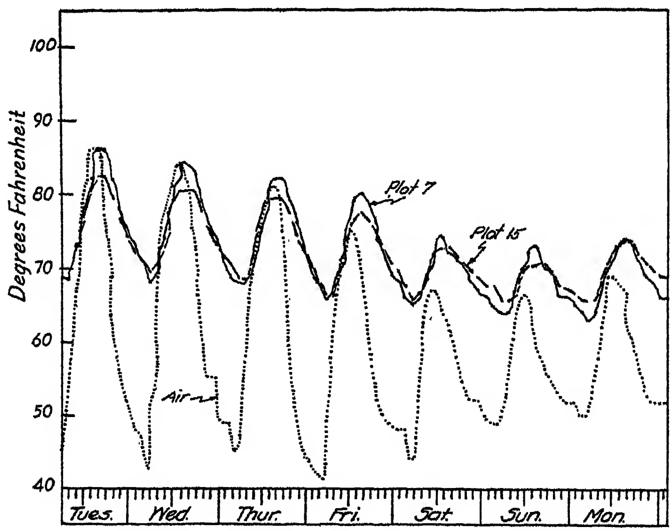

Fig. 5. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

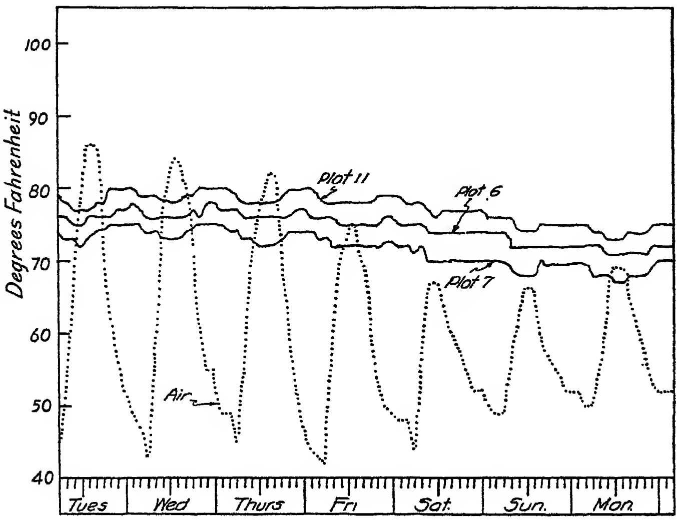

Fig. 6. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 11—Covered with Pabco Thermo-Gen nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214 perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

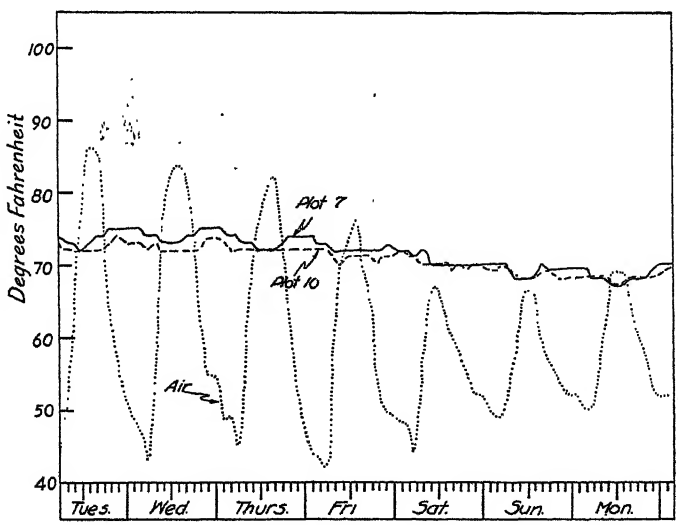

Fig. 7. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 10—Covered with Moistite Thermo-Gen perforated, small triangular slits, gray on both sides. Plot 7—Unmulched, cultivated once a month.

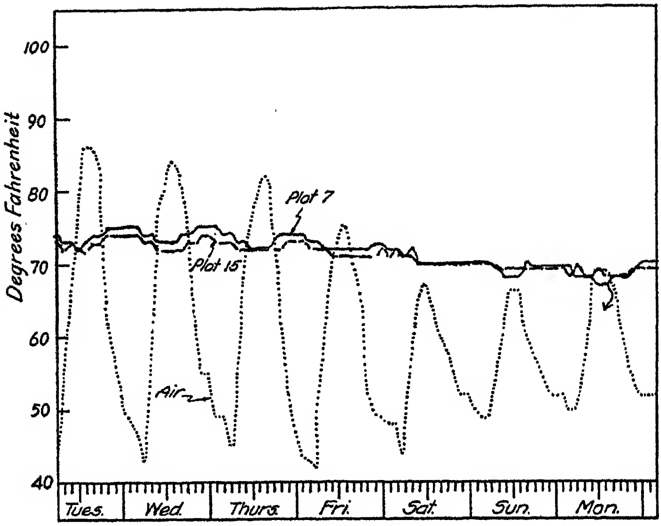

Fig. 8. Temperatures at 12 inch depth in mulched and unmulched plots and of air. Week of May 5–12, 1925. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

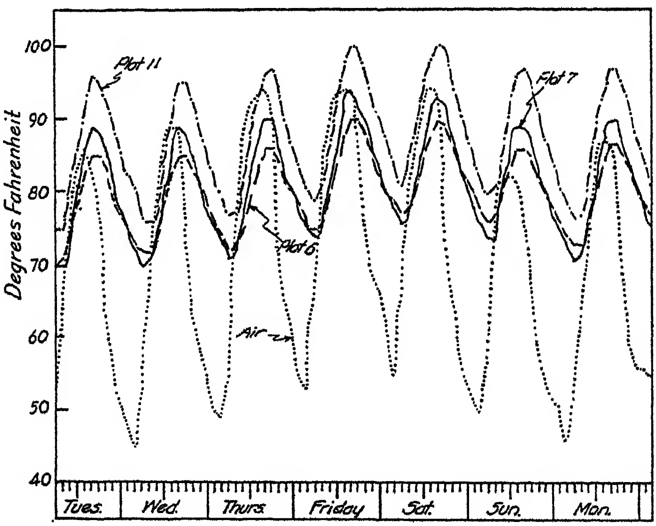

Fig. 9. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of June 9–16, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched cultivated once a month.

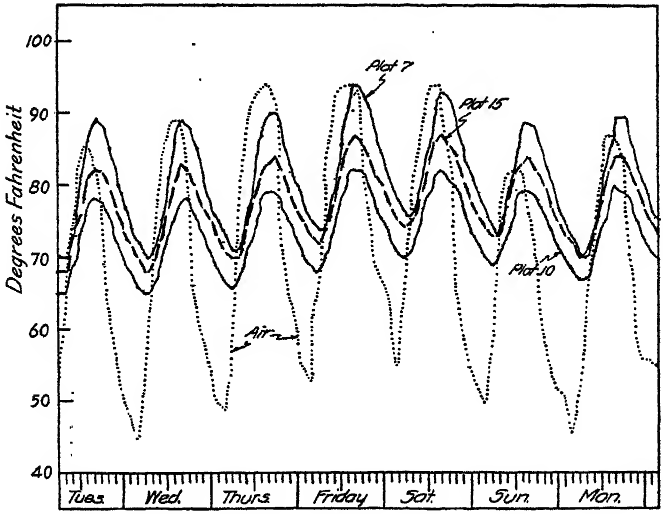

Fig. 10. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of June 9–16, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

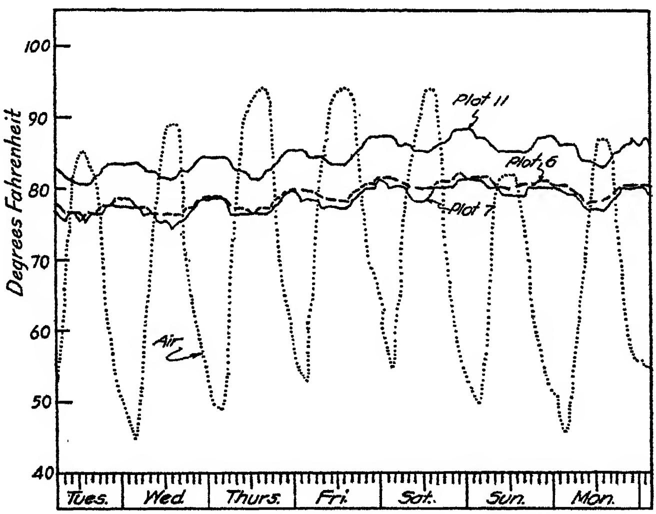

Fig. 11. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of June 9–16, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

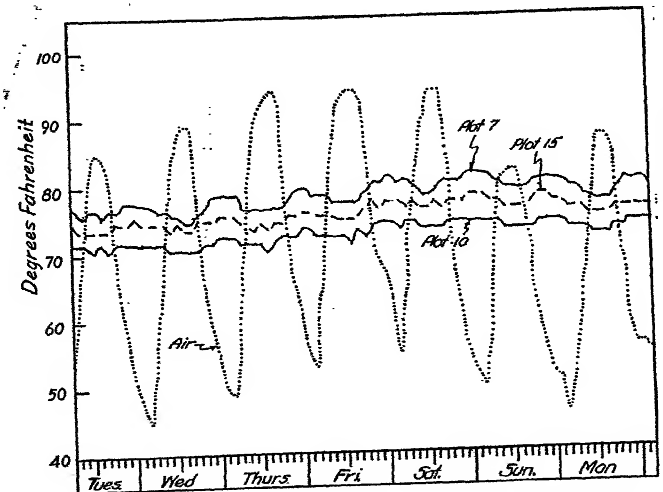

Fig. 12. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of June 9–16, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

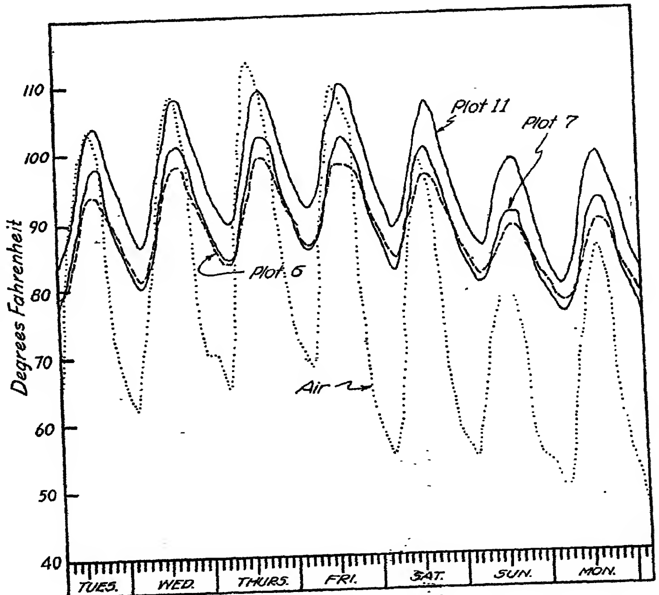

Fig. 13. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of June 23–30, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

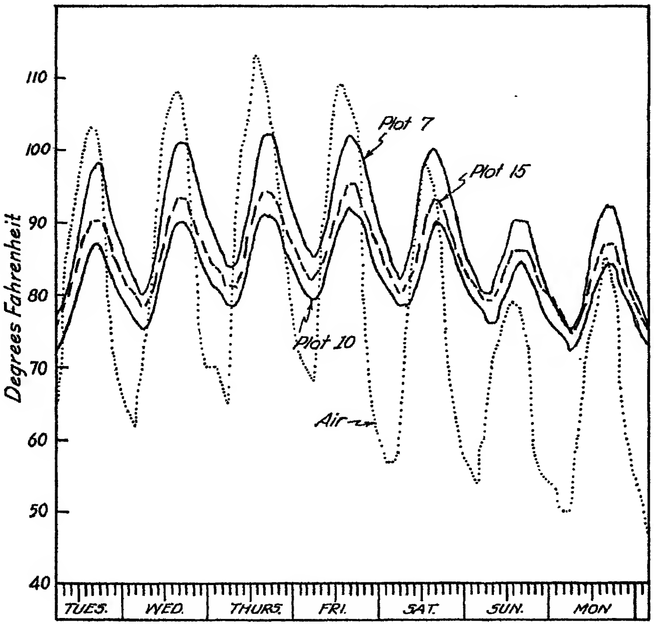

Fig. 14. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of June 23–30, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

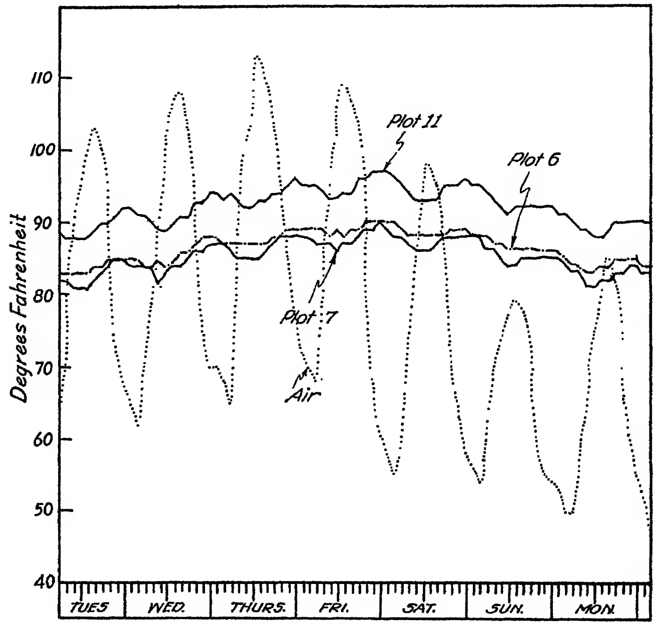

Fig. 15. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of June 23–30, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

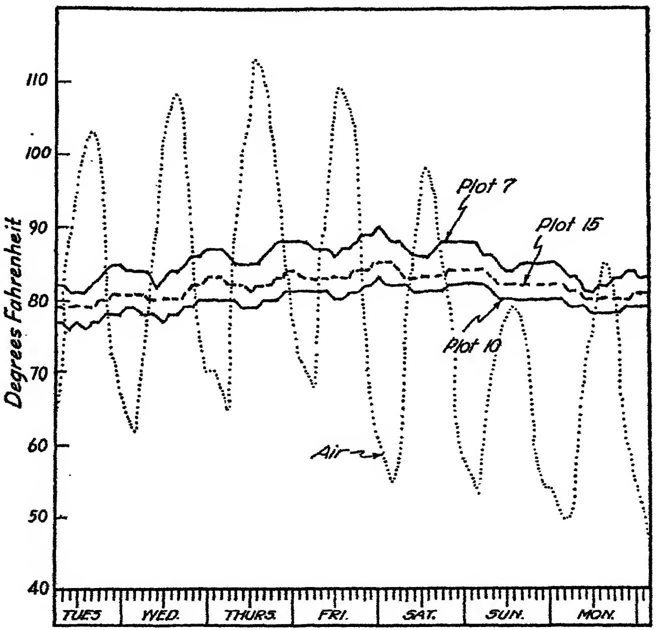

Fig. 16. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of June 23–30, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

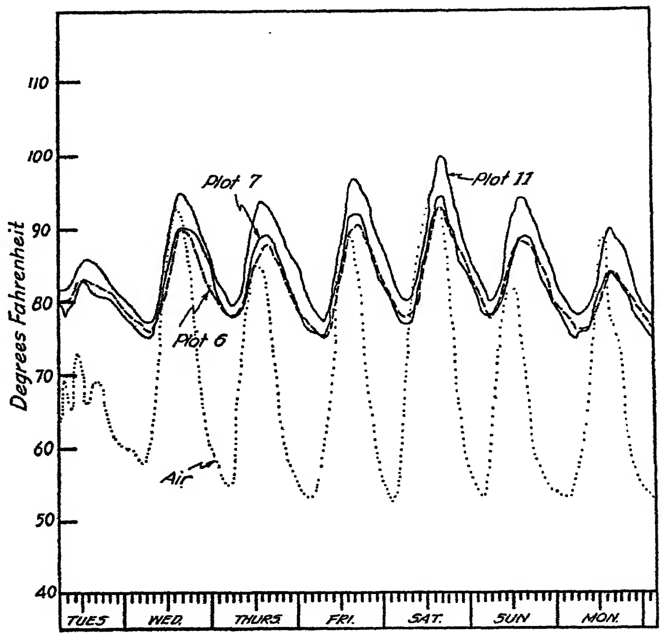

Fig. 17. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of August 11–18, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

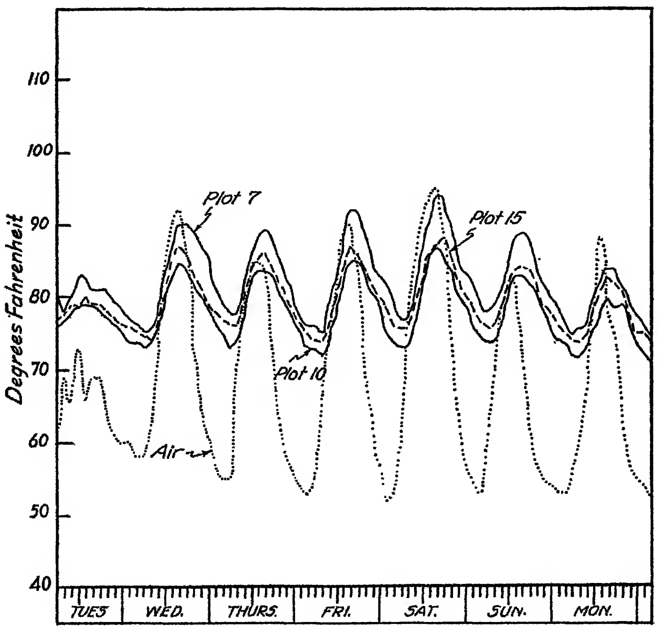

Fig. 18. Temperatures at 3-inch depth in mulched and unmulched plots and of air. Week of August 11–18, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

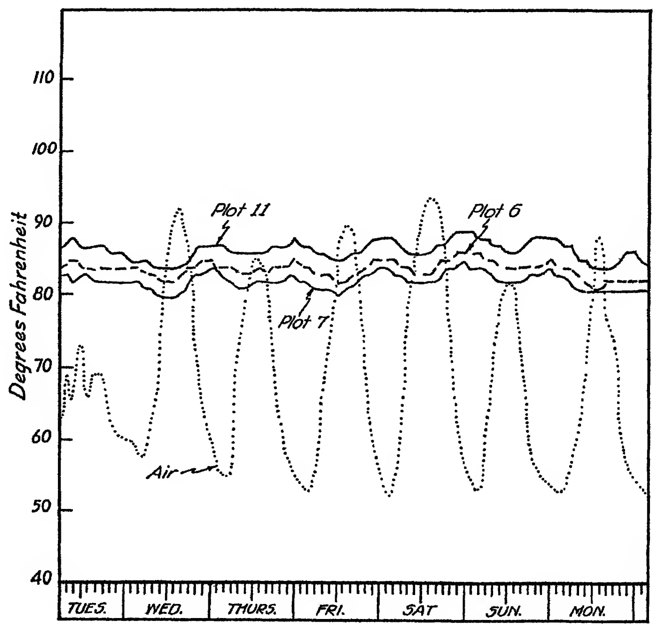

Fig. 19. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of August 11–18, 1925. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month.

The minimum temperatures at the 12-inch depth during these 5 weeks, occurred on the average of 5 hours and 5 minutes after the air minimum. The difference in time of occurrence of the minimum temperature in the different plots at the 12-inch depth does not appear to be significant.

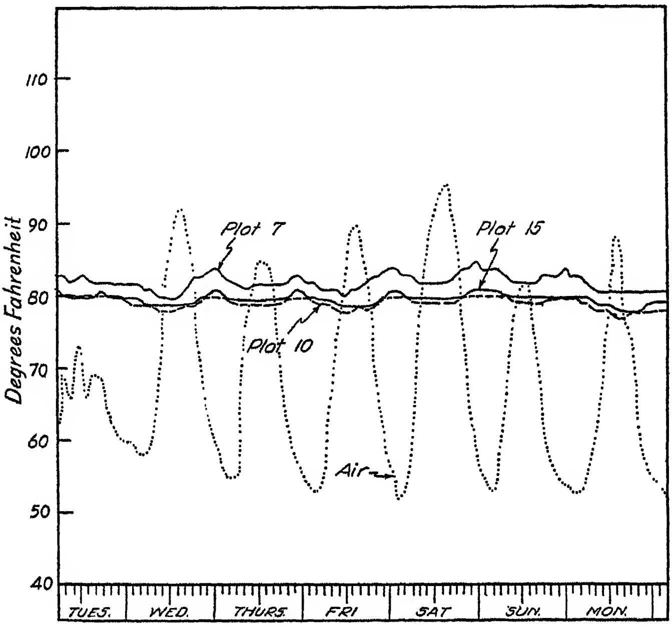

Fig. 20. Temperatures at 12-inch depth in mulched and unmulched plots and of air. Week of August 11–18, 1925. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 15—Covered with mulch paper plain, nonperforated, gray on both sides. Plot 7—Unmulched, cultivated once a month.

In figures 2 to 20 the temperature changes occuring at the 3-inch and 12-inch depths in the various areas are shown for 4 of the 5 selected weeks. Those for the period July 14–21 have previously been reported.[13]

Considering the temperature data for these 5 weeks when the character of the sky and wind direction varied, either by individual weeks or all together, or taking the averages by weeks for 147 days, the effect of the various surface treatments is always in the same order. The average day and night temperature for the 21 weeks, May 5 to September 30, 1925, at a depth of 3 inches was highest in the plot covered with black nonperforated paper, where it was warmer during

both day and night than in the cultivated plot. The area covered with black perforated paper had practically the same day temperatures but was slightly warmer at night than the cultivated plot. Under the gray perforated and nonperforated papers it was colder during the day and night than in the cultivated plot (fig. 21).

At the 12-inch depth the average temperatures for the 21 weeks were higher during the day and night, in the area covered with black nonperforated paper, and under the black perforated paper it was

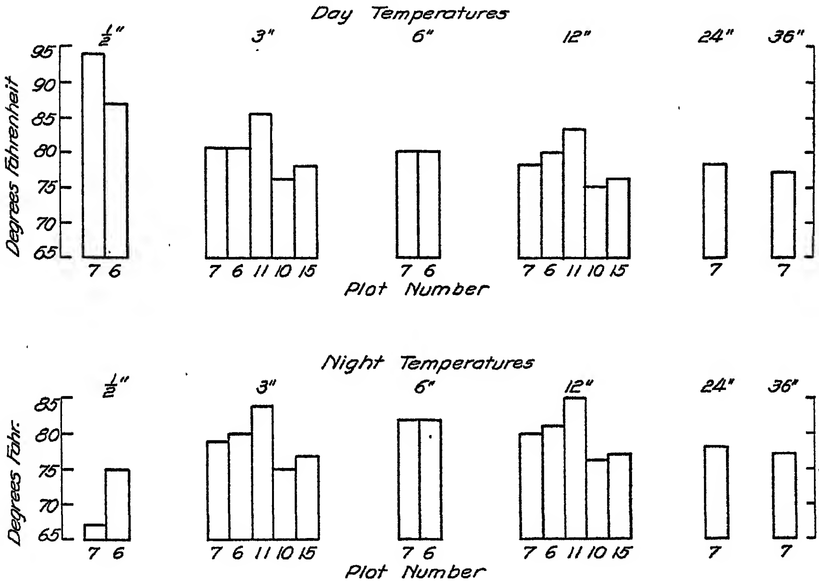

Fig. 21. Average day and night soil temperatures at various depths. May 5–September 30, 1925. Plot 6—Covered with Pabco Thermo-Gen No. 214, perforated, large triangular slits, black on both sides. Plot 7—Unmulched, cultivated once a month. Plot 10—Covered with Moistite Thermo-Gen, perforated, small triangular slits, gray on both sides. Plot 11—Covered with Pabco Thermo-Gen, nonperforated, black on both sides. Plot 15—Covered with mulch paper, plain, nonperforated, gray on both sides.

only slightly warmer during the day and night than in the cultivated plot. The areas covered with gray perforated and nonperforated papers were colder during the day and night than the cultivated plot.

In the cultivated plot, at a depth of 24 inches, the average day temperature was 78.4° F. This was higher than the average day temperature at a depth of 12 inches in the plots covered with gray perforated or nonperforated papers. The average night temperature at 24 inches in the cultivated plot was 78.2°, and this again was higher

than the average night temperature at a depth of 12 inches in the plots covered with the gray perforated and nonperforated paper mulches.

The greatest depth where soil temperatures were obtained was 36 inches and this only in the cultivated plot, where the average day temperature of 76.8° F was slightly higher than the average day temperature at a depth of 12 inches under the gray mulch papers. The average night temperature in the cultivated plot at a depth of 36 inches was 76.9°, which was slightly higher than the average night temperature at a depth of 12 inches in the plot covered with gray perforated paper and practically the same as the average night temperature at a depth of 12 inches in the plot covered with the gray nonperforated paper.

PAPER MULCH TRIALS IN 1926

As the result of the findings of the previous season, only two types of paper mulch were used in 1926. These two were alike save that one was nonperforated and the other was perforated. The papers were 36 inches wide, asphalt impregnated, and black on both sides. Each roll of both types contained 500 square feet and weighed 45 pounds. Type No. 1, known as Thermo-Gen No. 134, was nonperforated and cost $1.80 a roll. Type No. 2, known as Thermo-Gen No. 234, had small triangular perforations and cost $1.90 a roll. The perforations in Thermo-Gen No. 234 were cut sharper than in the black perforated paper used in 1925 and consequently there was less air movement through the slits.

In 1926 the flaps on the paper were not disturbed by rain as was the case in 1925. Under these conditions the marked differences found in 1925 between the perforated and nonperforated papers did not occur in 1926. The surface treatments used in 1926 are shown in table 2. On the plots which were only partially covered with paper mulch, the 3-foot-wide strips were so placed that the crop row was in the center of the paper strips. There was approximately as much bare bround between these strips as was covered by them.

Grain Sorghum Used as the Indicator Crop in 1926.—A grain sorghum (Yolo) was planted in this year. The areas which had not been cultivated or covered with paper mulch in 1925 were spaded 8 inches deep in December. The other areas were loose and mellow after the paper mulches were removed. All of the plots were hoed in February and again in April, 1926, and seeded between April 17 and 20.

Where the entire plot was covered with paper, a plank was placed on the paper and the seeding was done by working from this plank so as not to break the paper. Small holes were made 8 inches apart in the paper and soil, and the rows were spaced 4 feet apart; the seed of Yolo sorghum was placed $1\frac{1}{4}$ inches deep with two seeds per hole. On April 29–30, the plants were thinned out so that they were 8 inches apart in the row. Where the seed failed to germinate because of the dryness of the upper inch of soil or because cutworms or squirrels had destroyed the young plants, a second seeding was made. It was only necessary to reseed about 5 per cent of the area.

TABLE 2

PLOT TREATMENTS IN 1926 SEASON

Plot No.	Surface treatment
1, 2, 6	Unmulched, cropped to Yolo grain sorghum.
3, 4, 8	Partially covered with Thermo-Gen paper mulch No. 234, perforated, black on both sides; cropped to Yolo grain sorghum.
7	Unmulched, not cropped.
5, 9, 10	Covered completely with Thermo-Gen paper mulch No. 134, nonperforated, black on both sides; cropped to Yolo grain sorghum.
11, 12, 16	Covered completely with Thermo-Gen paper mulch No. 234, perforated, black on both sides; cropped to Yolo grain sorghum.
13, 14, 15	Partially covered with Thermo-Gen paper mulch No. 134, nonperforated, black on both sides; cropped to Yolo grain sorghum.

Moisture Determinations in 1926 Season.—In order to obtain information on the moisture distribution in the various cropped plots, soil samples were taken several times during the season from four locations in each plot. It should be remembered that at no time during this experiment has there been any water applied other than rain, but immediately before the seeding, a deep boring showed the soil to be moist to the water table, which at that time was at a depth of 22 feet. The first sampling in 1926 was made on April 1, prior to seeding, and moisture was determined by foot sections to a depth of 6 feet. Owing to rains totaling 5.37 inches between April 4 and April 9, it was necessary to resample on April 15. The third sampling was made on June 4, and the final sampling was made at harvest. The moisture content in the surface foot increased 2.19 per cent of the oven-dry weight of soil on the average between April 1 and April 15. In 1926 as well as in the previous season up to June 1, the immediate surface of the soil was more moist where black nonperforated paper was used than where there was no paper.

On June 4 samples of the surface foot were obtained by 4-inch layers and the second and third feet by foot layers. Expressing the

moisture content by foot sections, no marked differences were found on June 4. The averages of the combined three 4-inch sections of the surface foot under the paper mulches ranged from 13.77 per cent to 12.82 per cent of the oven-dry weight of soil; the second foot ranged from 19.44 per cent to 18.85 per cent. When considering only the surface 4 inches however, there was a difference of 3.69 per cent between the maximum and minimum. Up to June 4 the areas on which paper mulches had been placed contained an average of 2.89 per cent more moisture in the surface foot than in the unmulched cropped plots, based on oven-dry weight of soil. At the end of the growing season (September) the plots which had been cropped showed no significant difference in the moisture content of the 0–4 inch, 4–8 inch, and 8–12 inch sections of the surface foot. The total loss of moisture in the surface foot of the cropped plots during the growing season ranged from 12.58 per cent to 13.10 per cent based on oven-dry weight of the soil.

In the second foot between April 1 and April 15, the moisture content in the cropped plots was increased in all by an average of 1.99 per cent, and at harvest time the losses from all cropped plots were approximately the same, ranging from 11.50 per cent to 13.18 per cent based on oven-dry weight of the soil.

In the third foot the increase in the moisture content between April 1 and April 15 averaged 2.02 per cent, and at harvest the moisture losses from the third foot of the cropped plots ranged from 10.82 per cent to 12.87 per cent based on oven-dry weight of the soil.

Between the first and second sampling in April, the moisture content in the fourth foot increased by an average of 1.76 per cent, and the moisture losses up to harvest time, on the cropped plots, showed a greater range (9.70 per cent to 13.74 per cent based on oven-dry weight of the soil) than occurred in any part of the upper 3 feet of soil. This range in the fourth foot was due to the variation in texture of the soil which occurs at about this point. During the early part of April there was a slight increase in the moisture content of the fifth and sixth foot, and during the growing season the moisture losses were somewhat comparable to those occurring in the fourth. Judging from the condition of the crop, it appears that when the moisture content of the surface three feet of soil is reduced to 11 per cent and that of the 3–6 foot section is reduced to 8 per cent there is no readily available moisture for crops. Consequently, at the time of harvest, all of the readily available moisture had been used from the surface 6 feet of all the planted plots. The crop did not grow as large on the

unmulched plots as on those where the paper mulch was used, and this would naturally affect the rate at which the soil moisture would be used.

In summarizing the moisture losses by comparing the 0–3 foot and the 3–6 foot area, it was found that the unmulched cropped areas showed slightly less moisture loss in both sections. The moisture losses in the paper-mulched plots were in close agreement, but it is of interest that the order of the losses was always the same whether these were considered on the basis of 1-foot sections or 3-foot sections of the soil. The areas which were entirely covered with perforated paper had a greater moisture loss than those which were entirely covered

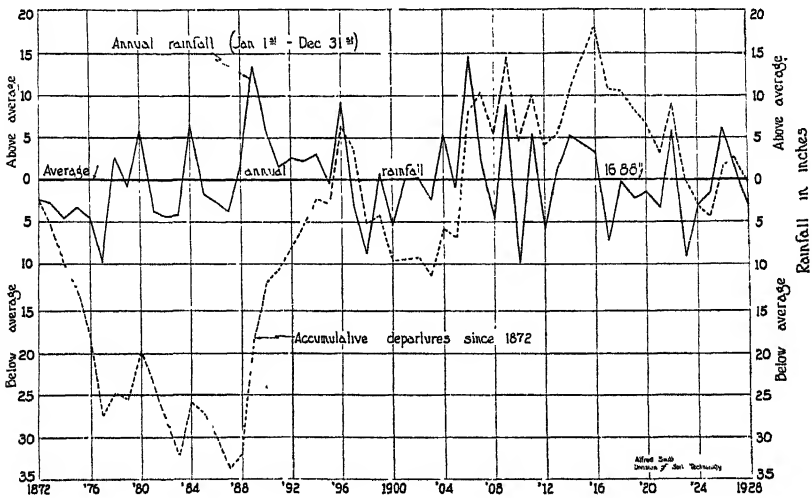

Fig. 22. Rainfall and accumulative departures at Davis, California, 1872 to 1928.

with nonperforated paper. Where the strips of nonperforated paper were used, the moisture losses were greater than where strips of perforated paper were used. In considering moisture changes as the growing season progressed, it is necessary to consider the possible shading effect of the crops during the latter part of the growing season. This is particularly noticeable in the soil-temperature studies discussed later.

A brief analysis of the rainfall records at Davis, California, are of interest at this point. Figure 22 shows the annual rainfall from 1872 to 1928 and the accumulative departures. The average annual rainfall for this period is 16.88 inches. It will be noticed that during the period 1924–1928, during which these experiments were being carried

on the annual rainfall each year and accumulative departures up to that time were close to the average annual rainfall line. The average seasonal (July 1–June 31) rainfall during this period of fifty-seven years is 17.03 inches. The average annual rainfall at Davis by ten-year periods has been as follows:

1879–1888	16.18
1889–1898	19.56
1899–1908	17.95
1909–1918	17.37
1919–1928	15.81

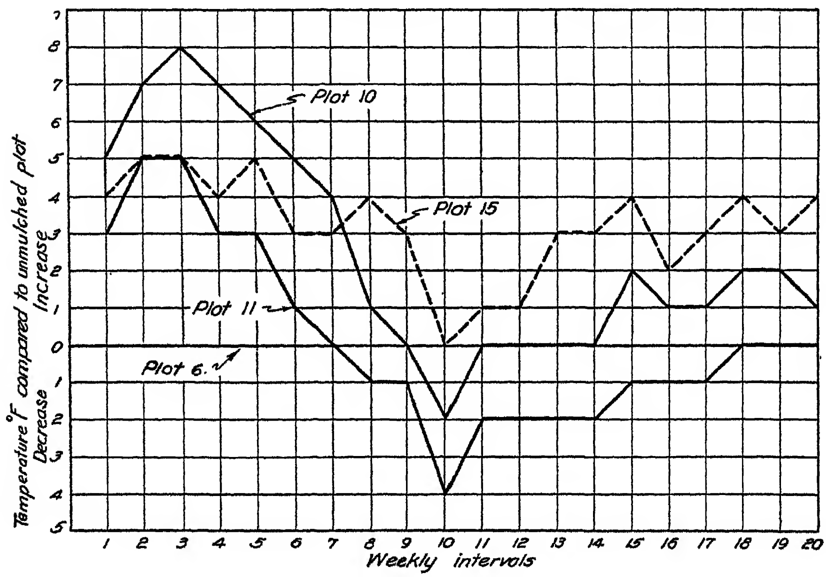

Fig. 23. Effect of paper mulch in the cropped plots at the 3-inch depth during the 1926 season (April 24 to September 2), as shown in the weekly day average temperatures in degrees Fahrenheit, higher or lower than the temperatures of the unmulched plot. Plot 6—Unmulched. Plot 10—Covered completely with Thermo-Gen No. 134, nonperforated. Plot 11—Completely covered with Thermo-Gen No. 234, perforated. Plot 15—Partially covered with Thermo-Gen No. 134, nonperforated.

Soil Temperatures in 1926.—Through a period of 20 weeks, from seeding time until harvest, soil temperatures were recorded from the soil thermometers in the cropped plots, which had remained in place and undisturbed since the previous year. The temperatures obtained at a depth of 3 inches in certain of the areas (figs. 23 and 24) show that where the nonperforated paper was used the weekly day and night average temperatures were higher than where the perforated paper

was used. In general, from the beginning of the growing season until the tenth week the difference in the cropped plots between the paper-mulched plots and the unmulched plot decreased because of the shading effect of the crop. The irregularity of the curve for plot 15 is probably due to the fact that at a depth of about 5½ feet in this area the soil is a sand with a much lower water-holding capacity and the Yolo did not continue to grow as well as in the plots where the texture at that depth was a fine sandy loam. The shading effect of the

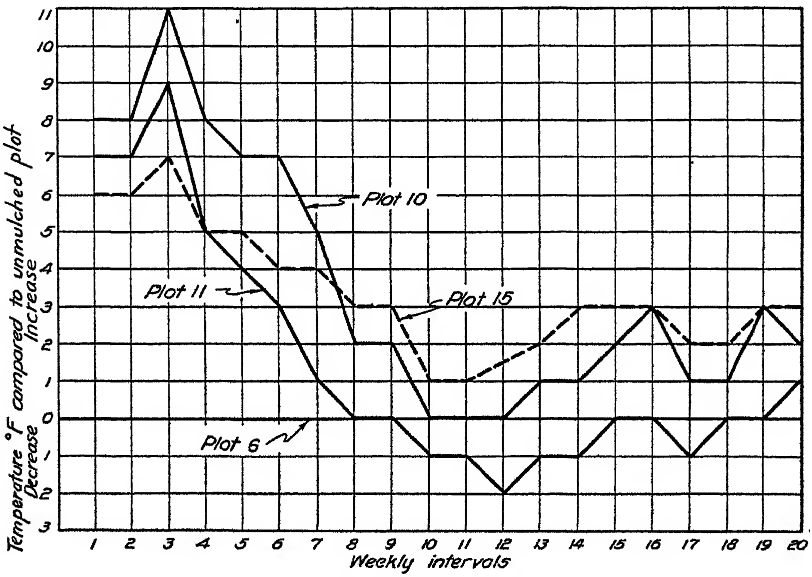

Fig. 24. Effect of paper mulch in the cropped plots at the 3-inch depth during the 1926 season (April 24 to September 2), as shown in the weekly night average temperatures in degrees Fahrenheit, higher or lower than the temperatures of the unmulched plot. Plot 6—Unmulched. Plot 10—Covered completely with Thermo-Gen No. 134, nonperforated. Plot 11—Completely covered with Thermo-Gen No. 234, perforated. Plot 15—Partially covered with Thermo-Gen No. 134, nonperforated.

Yolo was therefore not as pronounced on plot 15 as on the other plots. The plant roots in all plots extended below 6 feet in depth.

Towards the end of the growing season as the crop matured, the paper-mulched plots were warmer at the 3-inch depth than earlier in the season when the plants were in the thrifty green condition. In the area completely covered with nonperforated paper, the weekly day and night average temperatures at the 3-inch depth were in the early part of the season from 8 to 11° F higher than in the unmulched cropped plot. In 1926 temperature data indicate, as did those of the

previous season, that when the paper is perforated the entire width of the strip, the warming effect is not as great as when the paper is not perforated.

Figures 25 and 26 show the difference in the weekly day and night average temperature at a depth of 12 inches in the paper-mulched plots compared to the unmulched cropped plot, the general appearance of these figures being similar to figures 23 and 24. The weekly

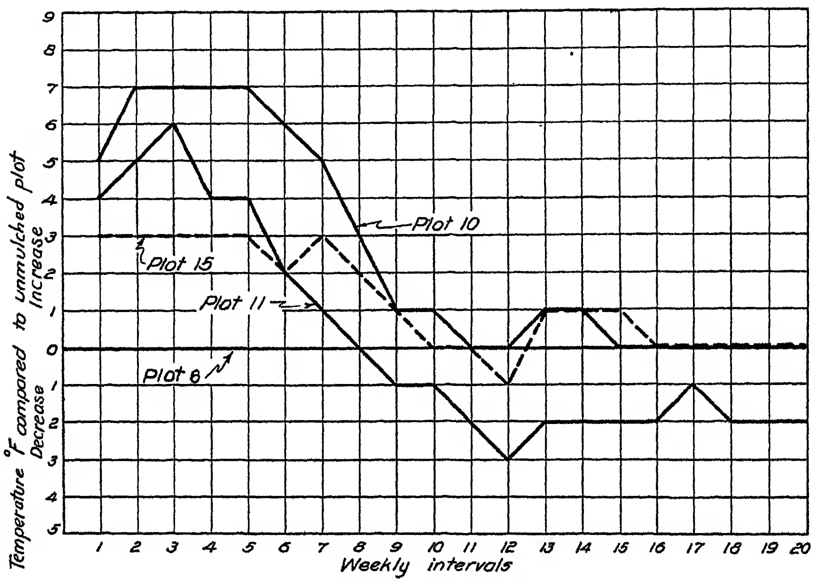

Fig. 25. Effect of paper mulch in the cropped plots at the 12-inch depth during the 1926 season (April 24 to September 2), as shown in the weekly day average temperatures in degrees Fahrenheit, higher or lower than the temperatures of the unmulched plot. Plot 6—Unmulched. Plot 10—Covered completely with Thermo-Gen No. 134, nonperforated. Plot 11—Completely covered with Thermo-Gen No. 234, perforated. Plot 15—Partially covered with Thermo-Gen No. 134, nonperforated.

day and night average temperatures at the 12-inch depth in the area completely covered with the nonperforated paper were sometimes as much as 7° F higher than in the unmulched cropped plot.

In general, before the crop had reached the height of 12 inches, the maximum and minimum soil temperatures at a depth of 3 and 12 inches in all the paper-mulched plots, occurred 20 to 40 minutes later than in the unmulched cropped plot. After the crop had attained a growth of over 12 inches in height these temperatures occurred from 12 to 20 minutes earlier in the paper-mulched plots than in the unmulched cropped plot.

Rate of Crop Growth in 1926.—As previously stated, the Yolo was planted on the various plots between April 17 and April 20, and by April 30 the height of the plants ranged from 1¾ to 2½ inches. The plots were all weeded on May 15. In the unmulched plots twice the time was necessary for weeding as in the areas that were partially covered with paper mulch, while on the plots which had been completely covered with paper, no weeding was necessary. By May 25,

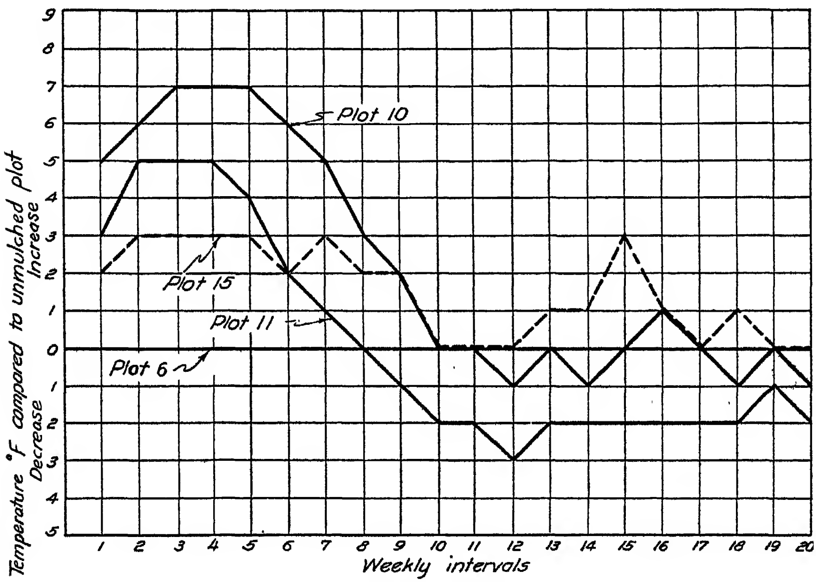

Fig. 26. Effect of paper mulch in the cropped plots at the 12-inch depth during the 1926 season (April 24 to September 2), as shown in the weekly night average temperatures in degrees Fahrenheit, higher or lower than the temperatures of the unmulched plot. Plot 6—Unmulched. Plot 10—Covered completely with Thermo-Gen No. 134, nonperforated. Plot 11—Completely covered with Thermo-Gen No. 234, perforated. Plot 15—Partially covered with Thermo-Gen No. 134, nonperforated.

where no paper mulch was used, the average height of the plants was 9 inches. The average height was 14 inches where the nonperforated paper was used, alike for plots entirely covered and for those only partially covered. The average height was 12 inches where the perforated paper was used, alike for entirely covered and for partially covered plots. On June 18, when the plants were nearly 9 weeks old, the least growth had been made on those plots on which there was no paper mulch and the best growth on those covered entirely with the black nonperforated paper. In the plots where the nonperforated paper was used, the plants made a better growth in the center of the

row than on the end, the difference in height averaging about 6 inches. In the plots covered with the perforated paper as well as those not covered with paper, the height of the plants in the rows was more uniform. In figure 27 the difference in the height of the plants in the row is apparent in the foreground (plot 10) where the area was completely covered with nonperforated paper.

By July 12 the plants growing on the unmulched plots showed only about one-third as many heads out as those growing on the plots completely covered with nonperforated paper, while in the other plots the plants had headed out from one-half to three-fourths as much as in

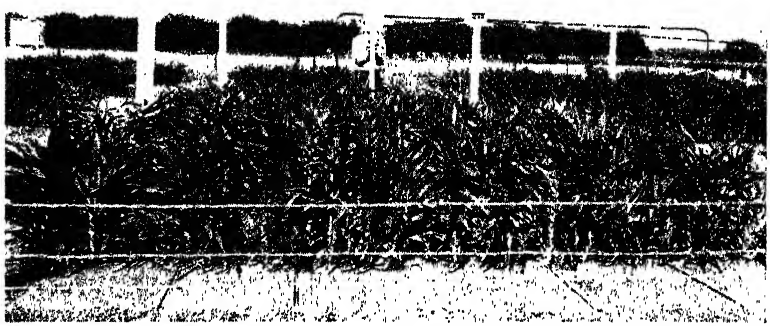

Fig. 27. A grain sorghum (Yolo) in the area completely covered with a black nonperforated paper mulch. June 28, 1926. The plants in the center of the plot made a better growth than those on the ends.

the area completely covered with nonperforated paper. By July 26, when the plants in all of the plots appeared to be completely headed out, the average height in the unmulched plots was 40 inches and in the paper-mulched plots 45 to 50 inches. The greatest height being in the area which was completely covered with nonperforated paper.

Birds were doing some damage to the crop by July 26, when the grain was in milk, and it appeared necessary to cover each row with cheesecloth and there was little damage from this source after that date. Figure 28 is a view of the plots after they had been covered with cheesecloth. The crop was harvested in early September, the yields for certain plots being reported by Gilmore.[1] In referring to plots 10 and 11, he showed that the average yields per plant of total produce (oven-dry matter) on these two plots was, for the nonperforated paper 28.4 per cent greater than the unmulched plot, and for the perforated paper 19.3 per cent greater. The average height

of the culms grown on the nonperforated paper plot was 21.1 per cent greater, and on the perforated paper plot 15.2 per cent greater than on the unmulched plot. The average weight of the grain per plant on the nonperforated paper plot was 10.3 per cent greater and on the perforated paper plot 3.1 per cent greater than on the unmulched plot. Smith,[1] using the averages of the triplicated plots, stated that where the soil was completely covered with nonperforated paper there was a material increase in the weight of heads, leaves, suckers, and grain.

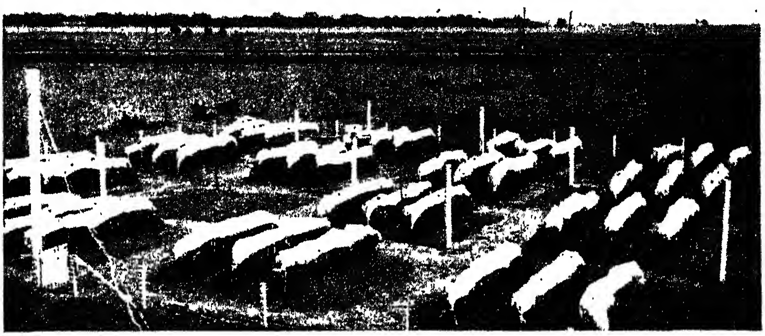

Fig. 28. Appearance of the plots after the rows had been covered with cheesecloth to prevent bird damage.

Placing the value of the yields on the unmulched plots at 100, the yields on the others were 126.8 for those completely covered with nonperforated paper; 110.7 for those completely covered with perforated paper; 105.8 for those partially covered with perforated paper, and 90.2 for those partially covered with nonperforated paper. Attention is again called to the fact that in certain sections of the plots partially covered with nonperforated paper, sand and gravel are present at a depth of 5½ feet, which may have influenced the yield adversely.

PAPER MULCH TRIALS IN 1927

All of the plots were spaded 8 inches deep on November 1, 1926, a total rainfall of 2 inches having fallen since the previous crop had been harvested in September. The thermometers were left undisturbed in the same locations as during the previous years. The plots were stirred by raking on February 1, and again on March 8, 1927, by which time a total of 15 inches of rain had fallen.

Potatoes Used as the Indicator Crop in 1927.—On March 8 seed tubers produced from tuber-indexed potatoes, and dipped for 1 hour in bichloride of mercury, was planted, and the temperature data herein recorded begin at sunrise on March 9. At the time of planting, the soil was in a good structural condition, being well granulated and having a favorable moisture content. Where the seed was large, it was cut into four to six portions with two eyes to each. The seed was planted 14 inches apart in rows 3 feet apart. The last plant in the row was 6 inches from the edge of the plot and the outside rows were 2 feet from the edge of the plot. Germination was 97.5 per cent and on April 25, the plants were rogued to one sprout to the seed and sprayed for aphis. The paper mulch was applied on April 27 and 28.

TABLE 3

EFFECT OF VARIOUS SURFACE TREATMENTS ON YIELD AND SHAPE OF POTATOES IN 1927

Plot No.	Surface treatment	Average weight of potatoes per plant	Average number of potatoes per plant	Average ratio of equatorial diameter to polar diameter
		grams		
6, 9, 13	Unmulched, cultivated once a month	87	1.76	0.78
1, 10, 14	Completely covered with nonperforated black paper mulch, Pabco Thermo-Gen No. 124	149	2.55	0.71
2, 11, 12	Completely covered with perforated black paper mulch, Pabco Thermo-Gen No. 224	127	2.00	0.72
3, 4, 15	9-inch strip of nonperforated black paper mulch, Pabco Thermo-Gen No. 124, on either side of crop row	105	1.76	0.74
5, 8, 16	9-inch strip of perforated black paper mulch, Pabco Thermo-Gen No. 224, on either side of crop row	95	1.68	0.77

Plot Treatments in 1927.—The treatment of the plots during this season is shown in table 3. Six plots were completely covered with paper mulch, while on six others a 9-inch strip of paper mulch was used on both sides of the crop row. Both types of paper mulch used were asphalt impregnated and coated and were black on both sides. The nonperforated black paper was Thermo-Gen No. 124, contained 900 square feet per roll, weighing 72 pounds, and costing $1.95. The perforated paper was Thermo-Gen No. 224, contained 900 square feet per roll, weighing 81 pounds, and costing $2.00. The perforations were 4 inches apart in the rows and the rows were 3½ inches apart. The perforations were U-shaped and 7/16 of an inch deep. Although there were several rains after the paper was laid, no pools were formed on any of the plots, all of the water having passed through the per-

forations or other openings in the paper. There was no permanent distortion of the papers as the result of the rain.

Moisture Determinations in 1927 Season.—As previous results indicated that the conservation of moisture by paper mulch was confined largely to the surface 4 inches of soil under the conditions (nonirrigated) of these experiments, soil samples from the surface foot were taken by 4-inch sections, and for the second to sixth foot inclusive by 12-inch sections. As the dry season advanced each year, it was noted that beads of moisture would appear on the under side of the nonperforated paper, and the surface 4 inches of soil was much more moist than the 4–8 or 8–12 inch sections. This would indicate that the accumulation of moisture was due to movement in the vapor phase with condensation on the paper. The condition was noted whether or not crops were being grown.

The first sampling for moisture determinations was made on March 21, the second on May 16, the third on June 11, after a rainfall of 0.47 inch which came on May 27–28, and the last sampling was made on June 20, after the potatoes were harvested. At the first sampling, the soil to the full depth of 6 feet in all the plots was found to be moist to field capacity except in the surface 4 inches in which it was about 2 per cent below field capacity. By May 16, in all the cropped plots except those completely covered with paper mulch, the moisture content of the surface 4 inches of soil averaged about 12 per cent of the oven-dry weight of the soil. In plots 1, 10, and 14, which were completely covered with nonperforated paper, the moisture content averaged 14.67 per cent and in plots 2, 11, and 12, which were completely covered with perforated paper, it was 13.83. On June 11 the moisture content of the surface 4 inches in all the plots was approximately the same (slightly less than 12 per cent). At harvest time, June 20, in all the plots except those completely covered with paper mulch, the moisture content of the surface 4 inches averaged 7.30 per cent, while in plots 1, 10, and 14, which were completely covered with nonperforated paper, it averaged 13.62 per cent, and in plots 2, 11, and 12, which were completely covered with perforated paper, it averaged about 13 per cent. The moisture changes in the surface 4 inches, that is, differences in the moisture content between the first and last sampling, show that in all the plots except those completely covered with paper, the loss of moisture was approximately the same (from 10.37 to 10.95 per cent based on the oven-dry weight of the soil) while in the plots completely covered with paper the loss was approximately one-half of this amount.

Between the first and last samplings there was a moisture loss in the 4–8 inch section of from 6.76 per cent to 8.46 per cent based on oven-dry weight of the soil, and the loss on any particular date was practically the same regardless of the surface treatment. In the 8–12 inch section by May 16, slightly greater losses of moisture had occurred in the plots completely covered with paper mulch than in the others, probably because, as will be shown later, the potato plants in these plots were more vigorous. By harvest time the moisture losses for the season at this depth ranged from 6.65 per cent to 7.58 per cent based on oven-dry weight of the soil. In the second, third, fourth, fifth, and sixth-foot depths there were no appreciable differences in the moisture content of the variously treated plots on any of the dates of sampling. In the uncropped plot, No. 7, which was hoed 4 inches deep once a month, the moisture loss in the surface 4-inch section was nearly 11 per cent based on oven-dry weight of the soil, in the 4–8 inch section slightly over 6 per cent, and in the 8–12 inch section nearly 5 per cent. In the second and third foot the losses were 3 per cent and 1 per cent respectively. The greatest changes, as can be seen from the above, were in the surface foot of soil. Slight changes in the moisture content of the soil of the second foot and deeper were usually found as the dry season advanced, probably owing to movement of moisture in the vapor phase.

Soil Temperatures in 1927.—Temperature data were obtained from planting time to harvest with the electrical resistance thermometers in the same locations (plots 6, 7, 10, 11, and 15) and under the same conditions as during the previous two seasons. As has been shown by the author,[14] distinct seasonal temperature changes occurred in the area under investigation to a depth of 3 feet.

From the temperature data the average day and night temperatures were determined in the cropped plots. At a depth of 3 inches, the soil temperatures in the various plots, up to the time when the paper mulch was put on, were within 1° F of each other. During the 7 weeks following the application of the paper mulch, where the 9-inch strips of nonperforated paper were used, the average day temperatures were only 1° higher than in the unmulched cropped plot. Where the nonperforated paper covered the entire plot, the average soil day temperatures were 6° higher, and where the perforated paper was placed over the entire plot they were from 3° to 4° F higher than in the unmulched cropped plot. No temperatures were obtained where the strips of perforated paper were used.

The average night temperatures at a depth of 3 inches in the various cropped plots, before the paper mulch was put on, were within 1° F

of each other. After the paper mulch was put on the average night temperature at the 3-inch depth, where the 9-inch strips of nonperforated paper were used, were 2° to 3° higher, and where the nonperforated paper completely covered the plot, it was 7° higher than in the unmulched cropped plot. Where the perforated paper was used, covering the entire plot, it was 4° to 5° F higher than in the unmulched cropped plot.

The average day and night temperatures at the 12-inch depth in the various cropped plots before the paper mulch was put on were usually within 1° F of each other. After the paper mulch was put on, in the plots with the 9-inch strips of nonperforated paper, the average

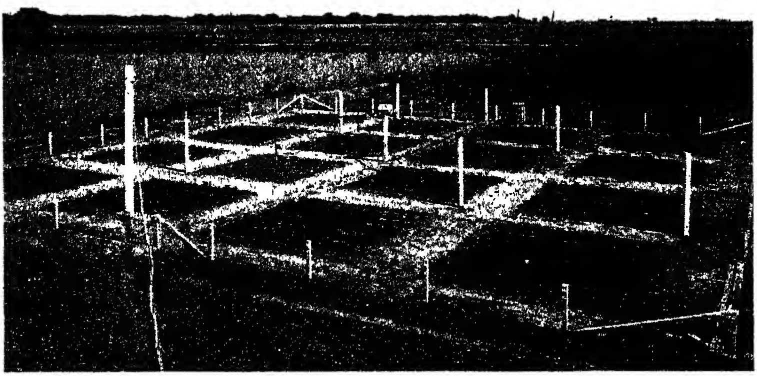

Fig. 29. The experimental area on May 12, 1927, showing potato plants after the paper mulch had been applied. The plots are numbered from right to left beginning with the bottom tier.

day and night temperatures were 1° higher, and where the nonperforated paper covered the plot completely, they were 5° higher than in the unmulched cropped plot. Where the perforated paper mulch completely covered the plot, the temperatures were 4° higher than in the unmulched cropped plot. After applying the paper mulch, the weekly average day and night temperatures at the 3-inch and 12-inch depths, in the various plots, ranged from 69° to 91° F.

Rate of Crop Growth in 1927.—The condition of the potato plants on May 12 is shown in figure 29. This view was taken about 2 weeks after the paper mulch was applied. On May 14 a careful survey of the condition of the potato plants showed that 95 per cent were thrifty, 3 per cent were weak, and the balance had failed to grow or had been removed because of serious injury from aphis. Between May 14 and harvest, it was necessary to remove more plants because

of fusarium wilt, aphis, and damage by gophers. By June 7, on the unmulched and on all of the partially covered plots, the potatoes were maturing and the leaves were starting to turn brown. On the six plots completely covered with paper, all the plants were green and apparently still growing. The earliest maturing plants were on the unmulched plots. The harvesting of the potatoes was started on June 20 and completed 2 days later, although 90 per cent of the plants in the plots completely covered with paper were still green. In the plots completely covered with nonperforated paper mulch, about 5 per cent of the potatoes were found on the soil directly underneath the paper. In all the other plots they developed below the surface of the soil. The potatoes produced by the end plants of each row were not included in the harvest data. Because some plants had been removed, the average number harvested per plot was 56, with a minimum of 51, and a maximum of 60.

The effect of the various surface treatments on the number of potatoes and the average weight of potatoes per plant is summarized in table 3.

With the cooperation of the late Dr. J. T. Rosa of the Division of Truck Crops, measurements of all the potatoes produced were made in order to determine what effect the various surface treatments had made on the shape of the potatoes. Two dimensions were obtained, *ED*, equatorial diameter, and *PD*, polar diameter, and the ratio $\frac{ED}{PD}$ determined (table 3). A study of this table will show that in the plots which were completely covered with paper mulch the potatoes were more slightly elongated than in the other plots. These are the plots having the higher soil temperatures. Similar observations have been made by others, as for instance, Jones, Johnson, and Dickson,[7] who in 1926 pointed out that "A temperature of 18° C gave the best or normal shape of potatoes, whereas at the higher temperatures, the tubers tend to become elongated and pear-shaped," and Werner[17] in 1929 found that "the low-temperatured tubers were more nearly round, having ratios of width to length varying from 1.00 to 1.25, whereas the similar ratios of the high temperature tubers generally were less than 1.00, indicating relatively a considerable degree of elongation." Werner also found that soil temperatures materially affected the color of the seed tubers but had no significant influence upon the productivity.

PAPER MULCH TRIALS IN 1928

All of the plots were spaded 8 inches deep on June 23, 1927, and again on February 10, 1928, and then hoed 4 inches deep on March 10, 1928. The thermometers were undisturbed and in the same locations as during the previous years.

Potatoes Used as the Indicator Crop in 1928.—On March 13 and 14, potatoes were planted in fifteen of the sixteen plots. The seasonal rainfall up to this time amounted to 11.03 inches. Seed of the White Rose variety, produced in Riverside County, California, was used because it was more uniform than the seed used during the previous season. The seed potatoes were dipped for 1 hour in bichloride of mercury, and since they were large, they were cut into from three to six pieces with two eyes to each. The distance of the planting and the depth was the same as during the previous season. On April 17 the plants were thinned to one sprout per seed and then hilled. On this date, after removing the weak plants, the stand was 96 per cent. The missing plants were replaced by transplanting from another part of the area, and the paper mulch was applied to the plots on May 4. No spraying for aphis was necessary during this season. The potatoes started to bloom on May 14.

Plot Treatments in 1928.—Four types of paper mulch were used during this season. The one designated as Asparagus Mulch paper weighed 100 pounds per roll of 900 square feet. The second was called Summer Mulch paper, Pabco Thermo-Gen No. 142, 900 square feet per roll, weighing 25 to 30 pounds. The third type was labeled Pabco Thermo-Gen No. 224 perforated, weighing 81 pounds per roll of 900 square feet. The fourth type was called Pabco Thermo-Gen No. 134, weighing 81 pounds per roll of 900 square feet. The cost of these papers ranged from $1.93 per roll for the Summer Mulch paper, the lightest, to $3.53 per roll for the Asparagus Mulch paper, the heaviest. All of these paper mulches with the exception of the Asparagus Mulch paper, were black on both sides. The latter was black on one side and gray on the other. The paper mulch was applied on May 4, each treatment being replicated on three plots. The Asparagus Mulch paper was placed with the black side up. It will be noted that only one paper, Pabco Thermo-Gen No. 224, was perforated. The three plots which were not mulched with paper were cultivated 4 inches deep once a month. The surface treatments of the various plots in 1928 are shown in table 4.

Moisture Determinations in 1928.—When the paper mulch was put on, the surface 6 feet of soil contained moisture up to field capacity. During the early season, beads of moisture collected on the under side of the paper mulch, particularly where the nonperforated types were used, but at harvest time there was no evidence of this.

TABLE 4

SOIL-MOISTURE CHANGES BASED ON DIFFERENCES BETWEEN THE MOISTURE CONTENT AT THE FIRST AND LAST SAMPLING IN THE UNCROPPED PLOT COMPARED TO THE CROPPED PLOTS IN 1928

Plot No.	Plot treatment	Moisture lost expressed as a percentage of oven-dry weight of soil					
		0–4 inches	4–8 inches	8–12 inches	12–24 inches	24–36 inches	36–48 inches
7	Uncropped plot	9.33	6.73	4.56	3.33	2.08	2.10
2, 6, 13	Unmulched, cultivated once a month, cropped	15.99	10.78	9.55	10.13	9.45	7.91
1, 10, 16	Asparagus Mulch paper, nonperforated, black side up, cropped	15.42	10.35	10.17	10.22	9.59	7.59
3, 11, 14	Pabco Thermo-Gen No. 142, nonperforated, black on both sides, cropped	15.96	10.93	10.31	10.10	8.98	8.92
4, 5, 15	Pabco Thermo-Gen No. 134, nonperforated, black on both sides, cropped	14.36	10.85	10.53	10.21	9.98	8.35
8, 9, 12	Pabco Thermo-Gen No. 224, large U-shaped perforations, black on both sides, cropped	14.55	10.46	10.26	10.55	9.19	6.21

In table 4 the moisture changes based on differences between the moisture content at the time of the first sampling, April 24, and the final sampling on June 25 are shown. These figures are the averages of four samples each from triplicated plots, and four for the uncropped area. No significant differences were found in the moisture losses of the cropped plots for the same depth at these dates of sampling. The greatest differences were in the 36–48 inch section.

Soil-moisture determinations were also made of the 0–6 inch and 6–12 inch sections on May 4 and June 4, at which dates soil-nitrate tests were also made. On May 4 there was no appreciable difference in the moisture content in the cropped plots for the surface foot of soil. On June 4 the moisture content of the 0–6 inch section in the paper-mulched plots averaged from 2.10 per cent to 2.56 per cent higher (based on oven-dry weight of soil), and in the 6–12 inch section from 0.65 per cent to 1.37 per cent higher than in the unmulched cropped plots. The perforated paper used in the 1928 season compared favorably with the nonperforated paper, for the flaps were not distended by any

heavy rains, and therefore there was no great circulation of air through the paper. At harvest time the plants in all of the plots were mature, while this was not true in the previous season.

Soil-Nitrate Determinations in 1928.—The nitrate content of the soil was determined by the phenol-disulfonic acid method at the times indicated above, from four locations in each plot and from a composite for each plot from the 0–6 and 6–12 inch sections. On May 4 the nitrate content (NO_3) of the 0–6 inch section in the cropped plots, those covered with paper mulch, and those unmulched, averaged from 10 to 17 parts per million of soil and 29 parts in the uncropped plot. One month later the nitrate content in the cropped plots in the 0–6 inch section averaged from 12 to 20 parts per million of soil, while in the uncropped plot it averaged 49 parts. On May 4, in the 6–12 inch section of the cropped plots, the nitrate content averaged from 15 to 18 parts and in the uncropped plot 30 parts per million of soil. One month later for the same depth in the cropped plots the nitrate content averaged from 3 to 8 parts, and in the uncropped plot 30 parts per million of soil. There were no consistent differences in the nitrate content of the various cropped plots. No nitrate tests were made of the growing plant material.

Soil Temperatures in 1928.—Temperature data were obtained with the electrical resistance thermometers in the same locations as during the previous three seasons. The temperatures for each thermometer were recorded continuously every 15 minutes and the average day and night temperatures were determined from these. Before the paper mulch was put on, the average temperatures at the 3-inch depth in the cropped plots were within 1° F of each other, while after the paper mulch was put on, they were sometimes 3° F higher under the paper than in the unmulched cropped plot. This was only true, however, during the first 4 weeks after the paper mulch was put on. After that time the differences were not so great. The weekly day and night average temperatures in the cropped plots at a depth of 12 inches were practically the same before and after the paper mulch was put on.

The average day and night temperatures at the 3-inch and 12-inch depth during this season (1928) were from 3° to 5° F higher than in 1927. As will be shown later, a more uniform and vigorous crop was obtained in 1928 than in 1927, which was doubtless primarily due to the better seed potatoes used (see fig. 30).

Crop Yields in 1928.—At harvest time, in June, the end plants and two of the weakest remaining plants in each row were discarded. The

total number of plants harvested per plot was 58. From table 5 it will be seen that yields as high as 315 grams of potatoes per plant were produced under the paper mulch, while during the previous season (1927) the highest yield per plant was 149 grams.

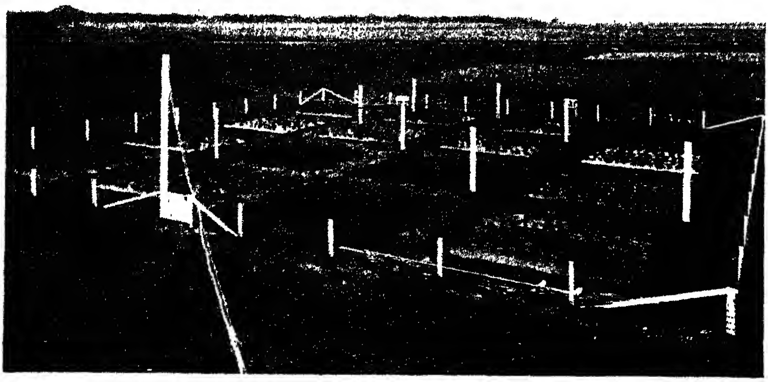

Fig. 30. Experimental area showing potato plants on May 8, 1928. The three unmulched cropped plots and the uncropped plot are readily discernible.

TABLE 5

EFFECT OF VARIOUS SURFACE TREATMENTS ON YIELD AND SHAPE OF POTATOES IN 1928

Plot No.	Surface treatment	Average weight of potatoes per plant	Average number of potatoes per plant	Average ratio of equatorial diameter to polar diameter
		grams		
2, 6, 13	Unmulched, cultivated once a month	272	4.10	0.72
1, 10, 16	Asparagus Mulch paper, nonperforated, black side up	299	4.42	0.69
3, 11, 14	Pabco Thermo-Gen No. 142, nonperforated, black on both sides	315	4.06	0 68
4, 5, 15	Pabco Thermo-Gen No. 134, nonperforated, black on both sides	294	4.52	0.68
8, 9, 12	Pabco Thermo-Gen No. 224, large U-shaped perforations, black on both sides	278	4.00	0.70

These data show that in those plots where the nonperforated or perforated paper mulch was used, the potatoes produced had a slightly lower ratio, $\frac{ED}{PD}$ than in the unmulched plots. The results obtained during this season on the $\frac{ED}{PD}$ ratio were comparable to those obtained during the 1927 season. No particular difference could be

determined in either the 1927 or 1928 season in the firmness of the potatoes produced under the various conditions. Werner[17] has pointed out, however, that potatoes produced under low temperatures are firmer than those produced under high temperatures.

Effect of Paper Mulches Used in Previous Years on Fenugreek Crop in 1929.—After the potatoes were harvested in June, 1928, the plots were spaded 8 inches deep and the soil left rough until November of that year when a winter cover crop of fenugreek was planted in all of the plots except No. 7, which had been kept free of plant growth since the start of these experiments. The rate of growth for the fenugreek was very slow owing to the abnormally cold spring. By March 11, 1929, it was only 6 inches high, but by April 11 it had reached a height of 22 inches. When turned under, in April, 1929, the green growth averaged 23,311 pounds per acre and there was no evidence from the rate of growth or total amount of green plant material produced in the various plots that the previous use of paper mulch on some of them had any effect on this crop of fenugreek.

SUMMARY

Paper-mulch experiments, extending over a period of four years, on a brown loam soil, at Davis, California, show that during the dry season of the year, under unirrigated conditions, the nonperforated black paper was the most effective in conserving moisture. This effect, however, was confined to the surface 4 inches of soil, and was due to condensation of water underneath the paper.

The greater proportion of the surface covered by paper, the more positive was the effect on the soil moisture, soil temperature, and crop yield. Black papers raised the soil temperatures, whereas gray papers reduced them. Where the paper was perforated the entire width of the strip, the soil temperatures were the same as or lower than the unmulched plots, according to the color of the paper used.

Very little weeding was necessary where the entire area of the plots was covered with nonperforated paper mulch, but where the perforated types were used, some weeding was necessary.

With a grain sorghum (Yolo) one season and potatoes for two seasons as indicator crops, it was found that paper mulches gave a slight increase in yield. Measurements indicated that the potatoes produced at higher temperatures, such as were induced by the use of black paper mulch, were more elongated and pear-shaped than those produced at lower temperatures.

The use of paper mulch except on small areas does not seem feasible at the present time because of the initial cost of the paper and its application, and the extra time necessary in planting and caring for the crop (spraying, etc.) in order to avoid undue injury to the paper.

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DOWNY MILDEW OF THE BEET, CAUSED BY PERONOSPORA SCHACHTII FUCKEL[1]

LYSLE D. LEACH[2, 3]

INTRODUCTION

The downy mildew, *Peronospora schachtii* Fuckel, on *Beta vulgaris* L. has been known in Europe since 1852 but was not observed in this country until 1911. Because of the supposed minor importance of this disease, no extensive studies of its prevalence and distribution had been made in America until 1929 when an epidemic of this disease destroyed over 40 per cent of the seed crop of the garden beet in central California.

Investigations showed that, although this disease has been known more than three-quarters of a century in Europe, comparatively little information was available regarding certain phases of the disease. Therefore, the pathogene was studied in regard to its life history, its host range, and the relation of environment to its attack.

THE DISEASE

Occurrence.—The downy mildew on *Beta vulgaris* was first described as *Peronospora schachtii* by Fuckel[16] in 1865 and was distributed as Fung. rhen. 1508. A slightly amplified description was

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[3] The writer wishes to acknowledge his indebtedness to Dr. J. B. Kendrick under whose direction these investigations were conducted, and to Dr. I. E. Melhus for helpful suggestions in the progress of the work and preparation of the manuscript.

published by the same author[17] in 1869. In 1872, Kühn[24, 25] presented a careful and accurate description of the macroscopic and microscopic symptoms of the disease, using the name *P. betae,* synonymously with *P. schachtii.* He stated that he first observed downy mildew of the beet in 1854 and that Schacht had observed the same disease sporadically in the years 1859 to 1861. The oospore stage of the causal fungus was not observed by Kühn[24] but he reported that by means of repeated experiments he had determined that *P. schachtii* overwintered as mycelium within the crown of the seed beets.

Prillieux[34] reported that downy mildew of the beet was first observed in France in 1852. He[33] first observed oospores of *Peronospora schachtii* in the leaves of diseased plants in 1882 and concluded that the fungus overwintered chiefly by means of these spores, rather than by hibernating mycelium as suggested by Kühn.[24]

Voglino[41] writing in 1899, agreed with Kühn as to the importance of hibernating mycelium and stated further that from such mycelium, condiophores and conidia may develop directly on the crown tissue of infected beets. He suggested that infected beets thrown on the refuse heap might provide inoculum for seedling beets during the following season. The production of conidiophores and conidia on the tissue of the beet crown has not been observed by other workers.

In England this disease was briefly studied in 1926 by Salmon and Ware,[38] who verified the presence of the mycelium within the beet crown, as shown by Kühn,[24] but were unable to find oospores. According to these authors, the first authentic report of the presence of *Peronospora schachtii* in England was in 1921, but Biffin[9] reported *P. schachtii* on mangels in England in 1913.

The first report of this disease in the United States was made in 1911 by Smith and Smith[40], who found infected sugar beets occurring to a limited extent in the coastal districts of California. Other reports from the same state have been made to the Federal Plant Disease Survey. Bensel[7] first recorded a severe outbreak of *Peronospora schachtii* on sugar beets in the Santa Clara Valley in 1927 and to a less extent in the Sacramento Valley the same year. He found from 1 to 66 per cent of the plants infected in the different fields and correlated the percentage of infection with the dates of planting and weather conditions. A similar outbreak, but of less intensity, occurred in 1925 in the same locality.

The only authentic report of *Peronospora schachtii* in other parts of the United States is that of a single infected plant of *Beta vulgaris* collected in New York on Long Island in 1917 and identified by H. S. Jackson (established through personal correspondence in 1929 with

Dr. Charles Chupp, Cornell University, Ithaca, New York). Gäumann[19] states that this fungus has also been collected in New Jersey and Minnesota. Communications from Dr. W. H. Martin of New Jersey and Dr. E. M. Freeman of Minnesota established the fact that there are no records of the collection of *Peronospora schachtii* in either state.

The geographic distribution of *Peronospora schachtii*, in so far as the writer has been able to determine, is as follows: Argentine, Belgium, Czecho-Slovakia, Denmark, Egypt, England, France, Germany, Italy, Japan, Palestine, Russia, Sweden, Switzerland, and United States (California and New York).

Fig. 1. Seedlings of *Beta vulgaris*, showing curling of the cotyledons caused by conidial infection of *Peronospora schachtii*.

Symptoms.—The disease exhibits several rather distinct stages of development, depending on the age and maturity of the host and on the ecological conditions. Symptoms of *Peronospora schachtii* infection on both young and old rosette leaves have been described by Kühn[24], Prillieux[33], and Voglino[41]. Salmon and Ware[38], and Hollrung,[22] described, in addition, the gross macroscopic effects of *P. schachtii* on the inflorescence. The symptoms on cotyledons of beet seedlings and on individual flower parts have not previously been reported.

When seedlings are infected while still in the cotyledon stage, the affected portions assume a color somewhat lighter than is normal for the variety, and the entire cotyledon usually curls downward (fig. 1). Under humid conditions a heavy coating of conidiophores and conidia appear on the upper as well as on the lower surface of the infected cotyledon and the young plants usually die from the effects of the disease. Cotyledon infection was readily produced by spraying with a conidial suspension under artificial conditions but has rarely been observed in field plantings.

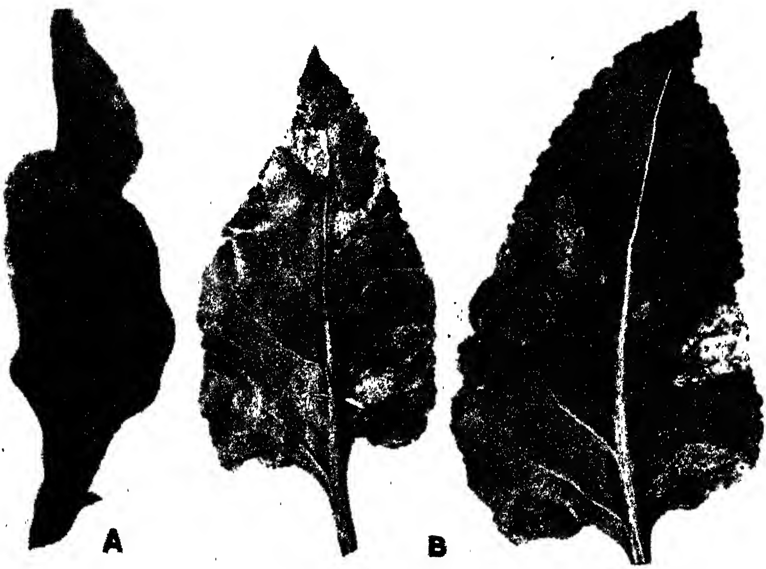

Fig. 2. *Peronospora schachtii* fructification on leaves of *Beta vulgaris*.

A. Conidiophores and conidia covering the lower surface of a young leaf. x4.

B. Isolated mildew lesions on older leaves.

A second type of infection occurs on the older leaves of the plants and presumably originates from air-borne conidia. The disease in this case manifests itself by isolated or sometimes coalescing spots of irregular shape, ranging in size from one to four centimeters in diameter (fig. 2*B*). These spots are differentiated from the healthy tissue by the lighter green color of the upper surface of the leaves and by the heavy coating of mildew fructification on the lower surface (fig. 2*A*). In this stage the effects of *Peronospora schachtii* on *Beta vulgaris*

bear a striking resemblance to those of *Peronospora effusa* (Grev.) Rab. on *Spinacia oleracea* L. During periods of extremely low humidity these spots on the beet leaves are sometimes surrounded by a narrow ring of pale red pigment. The symptoms described above are commonly observed in the fall-planted root beds (fields in which the seedling beets are maintained from the time of planting up to the age of three or four months) in the Sacramento Valley during the

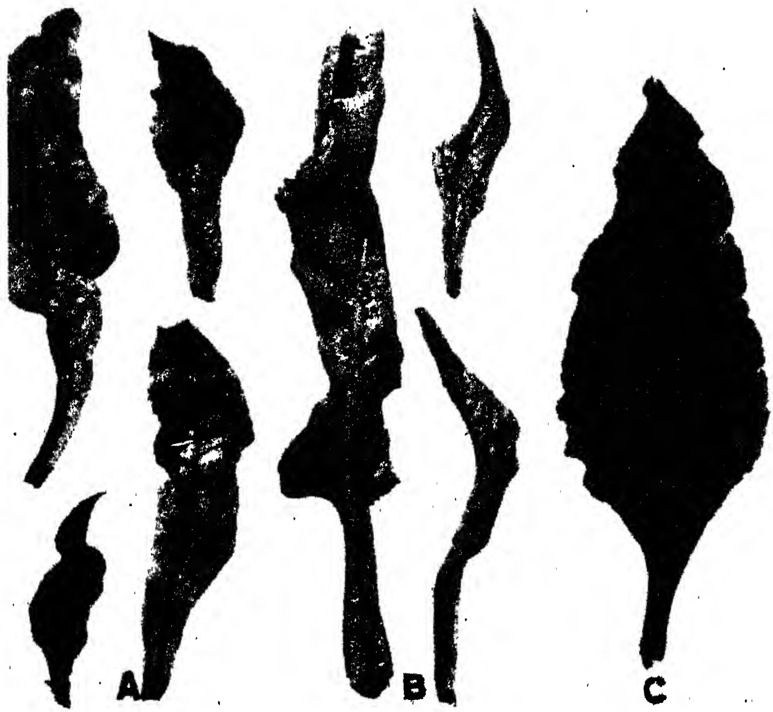

Fig. 3. Effects of *Peronospora schachtii* on young leaves produced in the center of the rosette on severely diseased plants of *Beta vulgaris*.

A. Leaves showing infection along petiole and basal portion of the blade.

B. Distortion of inner rosette leaves invaded by the fungus.

C. Healthy leaf.

months of October and November and in the seed-beet fields (fields devoted to seed production) during March and April. In the coastal regions of California, where the temperature is usually somewhat lower and the humidity higher during the spring months, this type of infection can be observed as late as June.

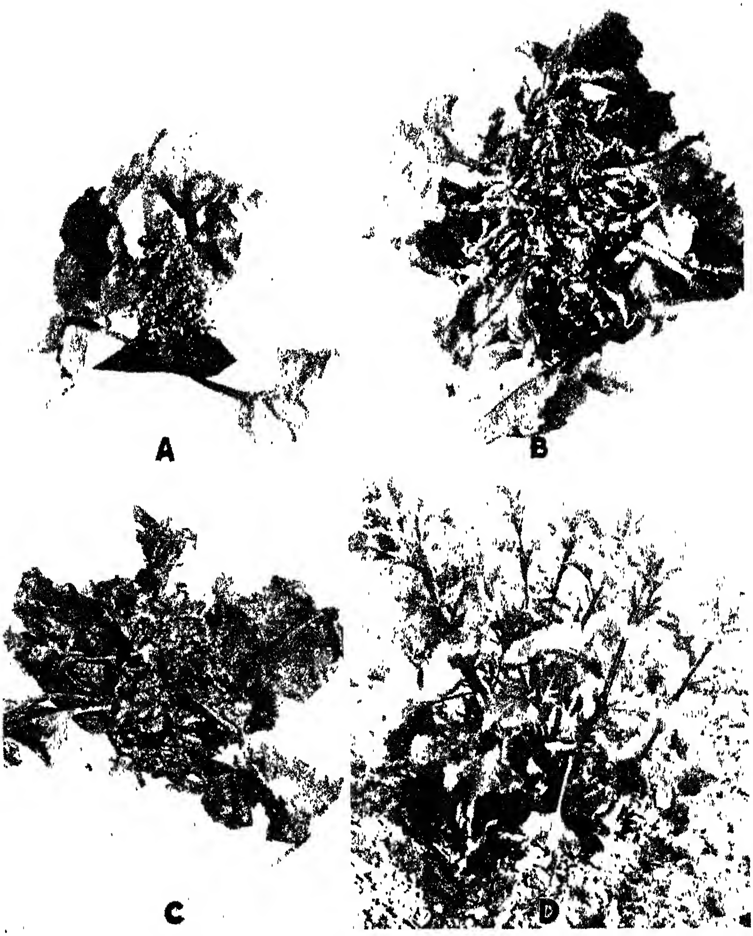

Fig. 4. Effects of *Peronospora schachtii* on mature beets.

A and *B*. Severe infection on beet plants showing the curling, distortion, and stunting of the young rosette leaves.

C. Small shoot developing from a severely infected plant.

D. Floral shoots on healthy plant of the same age.

Late in the fall, and especially after a period of heavy rainfall, the symptoms of the disease most frequently described in literature develop. The young rosette leaves of the beet are attacked by the fungus and either the entire new leaf is covered by the mass of conidiophores or merely the basal portion of the blade and the petiole (fig. 3*A*). Microscopic studies show clearly that there is a continuous mycelial connection between leaves infected in this manner and the crown of the beet. Usually all new leaves produced subsequently show mildew infection in their entirety (fig. 4*B*). Such leaves are always small, thick, and often curled downward at the edges (fig. 3*B*). In most cases the color of infected leaves is light green but in some fields there seems to be a rather high correlation between the presence of *Peronospora schachtii* and red pigmentation upon the younger leaves. Conidiophores and conidia cover the lower surface of the leaves and also may appear on the upper surface under humid conditions. The presence of the fungus apparently acts as a stimulus to the production of new leaves, since on infected plants there are usually from two to twenty times as many leaves (fig. 4*A* and *B*) as on healthy plants growing under the same conditions (fig. 4*D*). In nearly all cases the mass of infected leaves is surrounded by a number of fully developed and apparently healthy rosette leaves (Fig. 4*A*, *B* and *C*). This condition is commonly found in seed-beet fields during the late winter and early spring months and is also the condition commonly observed in the market gardens and sugar beet fields. When the disease becomes extremely severe the affected leaves are killed and then are invaded by numerous saprophytic fungi and bacteria. The decay which results usually spreads down into the beet crown and finally the entire plant is destroyed. Many missing hills result from this process in infested fields. When the first floral shoots develop on infected plants usually all the leaves formed on them are stunted, curled, thicker than normal and, in many cases, covered with conidiophores and conidia. As the main axis elongates, however, the primary cauline leaves are often normal in appearance, although the axillary flower shoots are dwarfed and distorted by the fungus attack (fig. 5*C*). The sepals of infected flowers and the bracts at the base of the flowers become swollen and assume a light green color. During periods of cool, moist weather, conidiophores and conidia develop in large numbers on the sepals, bracts, leaves, and even on the axes of the secondary branches of the inflorescence (fig. 6*A*). On the smaller secondary branches many swellings or blisters, often covered with *P. schachtii* fructifications, are found. By micro-

scopic examination it was observed that the tissue underlying such areas is permeated by non-septate mycelium and haustoria characteristic of *P. schachtii.*

Fig. 5. Effect of *Peronospora schachtii* on the beet inflorescence.

A. Infected beet inflorescence in which leaf production has largely replaced flower production.

B. Compact floral shoots bearing infected flowers.

C. Infected flower shoots produced in the axils of apparently healthy cauline leaves.

D. Severely injured inflorescence branch bearing a few mature seed balls.

E. Portion of the inflorescence of a healthy beet plant.

Because the branches of the inflorescence fail to elongate, the flowers and seed balls (aggregates of two or more flowers fused at their bases) remain grouped together and the entire inflorescence

assumes the appearance of a compact cluster (fig. 5*B*) as compared with a normal paniculate spike (fig. 5*E*). Suppression of flower parts frequently occurs, accompanied by increased leaf production which results in the formation of a structure resembling a witches' broom (fig. 5*A*). The infected portions of the plant appear bleached and the

Fig. 6. *A*. *Peronospora schachtii* infection on the bracts and flowers of secondary inflorescence branches.

B. Healthy branch of approximately the same age.

hypertrophy of the individual flower parts is evident. Although in the case of severe infection most of the flowers are destroyed it is not uncommon to observe on the same shoots apparently healthy seed balls and sterile flower remnants covered with fructifications of *Peronospora schachtii* (fig. 5*D*). Observations on material from infected plants revealed many mature, viable seed balls which still carried the conidiophores of *P. schachtii* upon the dry, corky sepals (fig. 7*A*).

Economic Importance in California.—In central California losses caused by *Peronospora schachtii* occur in three types of beet plantings: market gardens, sugar-beet fields, and fields of garden beets grown for seed production. Garden beets are extensively grown in truck gardens along the coastal region of the San Francisco Bay

Fig. 7. Effect of *Peronospora schachtii* on seed balls of *Beta vulgaris*.

A. Mature seed balls covered with dry conidiophores of *Peronospora schachtii*. x4.

B. Apparently disease-free seed ball. x4.

district where, because of the high atmospheric moisture and the absence of high temperatures, *P. schachtii* occurs throughout the entire year. However, the greatest damage occurs in the months of January, February and March, during which period the amount of marketable beets is often reduced 25 to 50 per cent by the disease.

Peronospora schachtii has been observed on sugar beets since 1911 in the fields of the coastal region. Severe damage has not been reported except in the years of 1925 and 1927 when, according to Bensel,[7] 66 per cent of the plants in some fields were infected. Unfortunately there is no record of the effect of the mildew attack on the ultimate yield in the fields mentioned above. In recent years sugar beet production in the Santa Clara and Salinas valleys has been largely discontinued, partly because of the severity of curly top, a virus disease transmitted by the beet leafhopper (*Eutettix tenellus* Baker). At the present time large acreages of sugar beets are being produced in the Sacramento and upper San Joaquin valleys. A detailed survey of many fields in this area was made during the past two years and in no case was downy mildew found in destructive amounts. In the spring of 1929, approximately one per cent of the plants in one field were found to be infected, while in other fields, either no diseased plants or only a few were found. It was observed that in the latter case a single plant was often severely infected apparently without any spread of the disease to neighboring plants.

When sugar beets are planted in late November or December in the Salinas valley, sufficient growth occurs before the spring migration of the leafhoppers to enable the plants to partially withstand the effects of the curly-top disease. In one of the trial plantings near Salinas, California, seeded in November, 1929, 73 per cent of the plants were found to be infected with *Peronospora schachtii.* Determinations made in the above-mentioned field indicate that the tonnage was reduced 17.3 per cent, the sugar percentage was lowered 1.46, the percentage of purity 4.96, and the total sugar yield was reduced 29.3 per cent, by the downy mildew epidemic. Since winter planting is becoming a commercial practice in the Salinas Valley, it is probable that downy mildew will become a more important factor in sugar beet production than is now the case.

Although no survey of sugar beet fields in southern California has been made by the writer, the disease is known to occur there, since specimens infected with *Peronospora schachtii* have been sent in for identification.

By far the greatest losses caused by this disease in central California occur in the fields devoted to the production of garden beet seed (fig. 8). In 1929 an estimate of the reduction in yield caused by downy mildew in the 600 acres devoted to beet-seed production in the lower Sacramento Valley, showed that 23 per cent of the acreage was abandoned as a total loss, 17 per cent suffered a 75–90 per cent

loss, 18 per cent a 25 per cent loss, and 42 per cent a loss of less than 5 per cent. A conservative estimate of the total reduction in yield of the 600 acres was 43 per cent. The actual loss caused by the disease averaged $100 per acre or a total of $60,000. During the season of 1930, the disease was present in most of the seed fields but the damage was less severe than in the previous year. The reduction in yield for the entire district was estimated at 25 per cent.

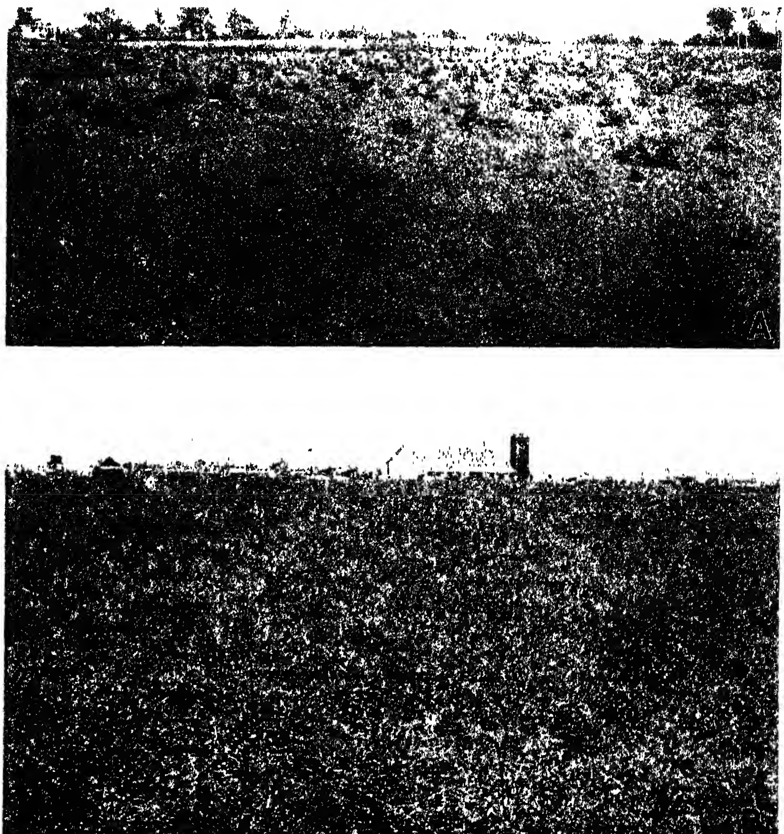

Fig. 8. *A*. Detroit Dark Red beet seed field in which 95 per cent of the plants were infected with downy mildew. Clarksburg, California, May 2, 1929. Yield: 214 pounds of seed per acre.

B. Detroit Dark Red beet seed field in which only 3 per cent of plants showed downy mildew injury. Clarksburg, California, May 24, 1929. Yield: 2,200 pounds of seed per acre.

Under central California conditions the methods of beet-seed production have been modified so that the beet plant, a true biennial, completes its life cycle within a period of ten to twelve months. During August or September the seed is planted in rows 15 inches apart in root beds where, forced by frequent irrigation, the plants produce stecklings (small beet roots used for commercial seed production) from one-half to two inches in diameter before the cool weather of November and December occurs. After the fall rains permit the preparation of fields, the beet plants are transplanted direct from the root beds to the seed fields where they are spaced from 36 to 40 inches apart. The transplanting operation is usually completed in December or January. During the winter months growth is confined chiefly to root development but in March the rosette leaves develop rapidly, followed in April and May by the formation of the floral axis. Usually the mature seed balls are harvested during July in the interior valleys and during August in the coastal regions.

The losses from *Peronospora schachtii* on garden beets grown for seed production are due: first, to injury on beet plants in the root beds before transplanting to the seed fields; second, to death of plants in the seed fields; and third, to reduction in yield of the infected plants which survive.

Peronospora schachtii infection on beet seedlings in the root beds, while it does not severely injure the individual plants, is important in relation to the development of the disease in the seed fields. Seedlings may be weakened by the severe attack of *P. schachtii* on the leaves in the early stages of development, but after the fungus becomes established in the young rosette leaves the effect on the plant is much more marked. Such plants are practically worthless for transplanting and are a dangerous source of infection to nearby healthy plants. Observations have shown that downy mildew in the seed fields can, in nearly all cases, be traced directly to root bed infection. For this reason entire root beds are sometimes discarded when the percentage of infected plants is high, rather than risk transferring diseased plants to the seed fields.

During the spring months, *Peronospora schachtii* produces its most severe injury on the seed beets. Individual plants are so severely attacked that the rosette leaves of the plants become a stunted and distorted mass (fig. 4*B*). The death of certain of the leaves apparently provides an entrance for numerous saprophytic fungi and bacteria, which probably assist in completing the destruction of the plants. The reduction in stand in many cases constitutes the greatest single loss

from this disease. Infected plants which survive until warm weather occurs usually produce poorly developed inflorescences, and the amount of salable seed is always less than that produced by disease-free plants.

In order to secure information on the dissemination of *Peronospora schachtii*, a study of the disease as it appeared in root beds was undertaken. Ten root bed plantings were kept under observation to determine, as far as possible, how many original infections occurred, and the rate of spread of the disease in the field.

During the fall of 1929 the rainfall in the Sacramento Valley was unusually light, less than 0.15 inches having fallen before December 8. Conditions were relatively unfavorable, consequently, for the development and spread of the mildew, making it possible in the case of one isolated field to trace the development of the epidemic from its origin. This field of 1¾ acres was planted to the variety Detroit Dark Red on August 19, 1929, with seed of unknown origin. Observations made on August 28 and September 20 failed to reveal any infected plants but on October 10 one plant was found with severe *Peronospora schachtii* infection on several of the older leaves. The conidial mass on some lesions was very dark and beginning to dry up, indicating that infection had occurred at least two weeks before the date of observation. Early stages of infection were found on about 25 per cent of the plants within a radius of eight feet from the heavily infected plant mentioned above (fig. 9*A*). A careful survey of the remainder of the field, however, failed to reveal any other diseased plants. In order to determine the rapidity of spread, observations were made at intervals of one week throughout October and November. During this period the only moisture available for conidial germination on the leaves was dew, which however, was abundant throughout most of the period because of the surface irrigation practiced.

By October 18 the symptoms of disease were observed to have spread a distance of 90 feet, or entirely across the narrow field, consisting of five plant beds (fig. 9*A*). By October 25 the downy mildew had spread over one entire end of the planting and a small infected area appeared in the opposite end of the field (fig. 9*B*). As in the other case, one heavily infected plant was surrounded by others bearing evidence of lighter and more recent infection. Because of the distance and the absence of the disease between the two areas, the second is believed to be another case of original infection. It is possible, however, that it may have resulted from wind-blown conidial infection from the other end of the field, some 450 feet distant.

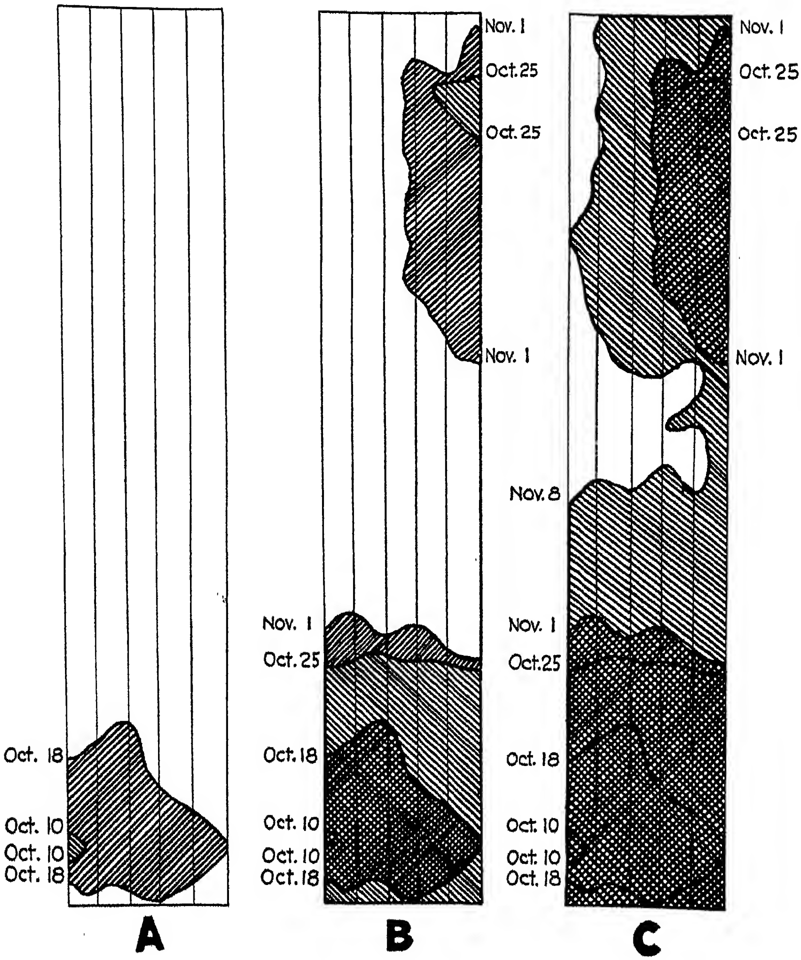

Fig. 9. Successive development of an epidemic of *Peronospora schachtii.*

A. Beet root bed showing areas found to be infested on October 10 and 18, 1929.

B. Same root bed showing areas invaded up to November 1, 1929.

C. Same root bed showing the spread of the disease from October 10 to November 8, 1929.

By November 1 both areas were somewhat enlarged but maintained their identity. One week later the two areas had coalesced along one side of the field and only a small area in the center and along one side remained free from infection (fig. 9*C*). After this date heavy frosts frequently occurred, and either this fact or the stage of development of the beet leaves seemed to be unfavorable for the spread of the disease. No new infested areas were observed on November 15 and the infection on many of the plants appeared to be less severe than formerly. Up to this time all the downy mildew lesions, with a few exceptions, occurred as isolated areas on the outer, well-developed leaves. Soon after the advent of the late fall rains, heavy infection of the inner rosette leaves could be observed, while the first type of infection practically disappeared. The greatest number of plants bearing the second type of infection were found in the areas in which the disease had appeared at an early date.

These observations indicate that infection may appear on seedling beets in the field within a month after their emergence. In the case of the field studied, all of the infections could be accounted for by wind-blown conidia except two, which were considered to be original infections. This fact explains how an epidemic covering an entire field may result from a few cases of successful seed transmission or oospore infection. Because of the rapidity of the spread even under relatively unfavorable conditions, it is evident that any attempt to control downy mildew in beet root beds by spraying or eradication must take into consideration the fact that the limits of infection at any given time extend considerably beyond the area showing visible symptoms of the disease.

HOST RANGE

Peronospora schachtii has been reported on the following hosts: sugar beets, garden beets, mangels (*Beta vulgaris*), and wild beets (*B. maritima* L.). In addition Ravn[35] reports *Peronospora schachtii* on the "Bladbede" while a popular article from Egypt[2] refers to the presence of spinach mildew (*P. effusa*) on Swiss chard. In May 1929, a downy mildew was observed on Swiss chard (*B. vulgaris* var. *cicla* L.) in the experimental plot at Davis, California. The fungus resembled *Peronospora schachtii* morphologically and readily infected garden beet seedlings, while *P. schachtii* conidia from garden beets infected Swiss chard seedlings under similar conditions. Natural infection of Swiss chard was later found on two occasions in the market garden section south of San Francisco. In repeated trials the

writer failed to produce infection on seedlings of Swiss chard with conidia of *P. effusa* from *Spinacia oleracea*, while on the other hand, Swiss chard seedlings were readily infected by conidia of *Peronospora schachtii.* From this evidence it appears that Swiss chard is susceptible to *P. schachtii* but not to *P. effusa* from *Spinacia oleracea* in California.

Since little information concerning the host range of *Peronospora schachtii* was available it seemed desirable to test the relative susceptibility of varieties of garden beets, sugar beets, and mangels, as well as to test the susceptibility of species of *Beta, Spinacia,* and *Chenopodium.*

All the plants to be tested were inoculated while in an early seedling stage, by spraying them with a conidial suspension. The seedlings were then incubated at 8° C for 48 hours in a saturated atmosphere, a temperature shown later in this paper to be favorable for infection. At the end of this time the seedlings were transferred to moist chambers on a greenhouse bench. On susceptible plants, sporulation occurred five or six days after inoculation and after eight days a record was taken of the percentage of plants showing the presence of conidiophores and conidia. It was found that the age of the seedlings greatly influenced the percentage of infection, the older plants being less susceptible. For that reason all comparisons are based on results obtained with young seedlings and these are divided into two age classes, the first from 11 to 20 and the second from 21 to 30 days after planting.

The investigations reported in table 1 were conducted between November 1929 and March 1930. The results with each variety represent the compilation from two or more trials. Eight varieties were inoculated at one time and seedlings of the variety Detroit Dark Red were included with each set so that a comparison of the separate trials could be made. Since over 90 per cent of the 11 to 20-day-old seedlings of the variety Detroit Dark Red, and over 80 per cent of those from 21 to 30 days of age were infected in every case, the results of all trials were brought together in one table.

Twelve commercial varieties of garden beets were tested and all were found to be susceptible in the seedling stage. There were, however, some differences and from these trials it appeared that the varieties Crosby's Egyptian and Detroit Dark Red were the most susceptible, while the varieties Long Smooth Blood and Crimson Globe were relatively resistant. It seems probable from the few trials that have been conducted that older plants of certain varieties may be markedly resistant to the attack of this fungus.

All of the nine mangel varieties tested showed some infection, but the variety Long Red seemed to be the most resistant. However, the number of seedlings of this variety was too few to permit of definite conclusions. Two sugar beet varieties, Kleinwanzleben and an unlabeled commercial variety were not as susceptible as the majority of garden beet varieties, but were readily infected in the seedling stage.

TABLE 1

COMPARATIVE SUSCEPTIBILITY OF CULTIVATED VARIETIES OF BETA VULGARIS TO PERONOSPORA SCHACHTII

Common name	Variety	Inoculated 11 to 20 days after planting: Total plants	Number infected	Per cent infected	Inoculated 21 to 30 days after planting: Total plants	Number infected	Per cent infected
Garden beets	Crimson Globe	75	52	69	103	64	62
	Crosby's Egyptian	89	88	99	136	131	97
	Detroit Dark Red	515	499	97	307	270	88
	Early Blood Turnip	101	97	96	92	56	61
	Early Eclipse	109	105	96	49	37	76
	Early Model	97	95	98	50	35	70
	Early Wonder	127	91	72	....	....	....
	Extra Early Egyptian	170	167	98	83	71	86
	Fireball	107	104	97	99	85	86
	Half Long Blood	95	87	92	172	101	59
	Half Long Special	183	134	73	182	147	81
	Long Smooth Blood	229	119	52	159	64	40
Mangels	Danish Slugstrup	132	117	89	169	125	74
	Giant Half Sugar Rose	225	191	85	109	71	65
	Golden Giant Intermediate	225	164	73	135	82	61
	Golden Tankard	86	80	93	83	50	60
	Long Red Mangel	22	5	23	27	3	11
	Mammoth Long Red	183	159	87	231	72	31
	Red Eckendorf	115	102	89	160	130	81
	White French Sugar	90	80	89	144	94	65
	White Sugar Rose Top	84	75	89	125	112	90
Sugar beets	Commercial sugar beets (variety unknown)	49	38	78	83	37	45
	Beta vulgaris var. *plantaginifolia*	200	86	43	85	3	4
	Kleinwanzleben	128	76	59	220	121	55
Swiss chard	Giant Lucullus	171	55	32	148	27	18
	White Silver	223	156	70	118	58	49

A sugar beet selection designated as *Beta vulgaris* var. *plantaginifolia* Zalenski was found to be resistant, especially when exposed to infection after the first true leaves had developed. It is interesting to note in passing that this selection of sugar beets was reported by

Shevchenko[39] as highly resistant to infection with *Cercospora beticola* Sacc. Both varieties of Swiss chard were susceptible to downy mildew, but Giant Lucullus showed more resistance.

Ten *Beta* species were inoculated by spraying them with conidia from garden beets and all became infected (table 2). Of those tested, *Beta bourgaei* Coss. and *B. patula* Soland. were apparently the most resistant. Samples of *B. maritima* obtained both from Europe and California were exposed to infection and over 70 per cent of the seedlings were infected in each case. *Chenopodium album* L. and *C. murale* L. were repeatedly sprayed with conidial suspensions but no infection occurred.

TABLE 2

COMPARATIVE SUSCEPTIBILITY TO INFECTION WITH PERONOSPORA SCHACHTII OF WILD SPECIES OF BETA AND CHENOPODIUM

Genus and species		Inoculated 11 to 20 days after planting			Inoculated 11 to 20 days after planting		
		Total plants	Number infected	Per cent infected	Total plants	Number infected	Per cent infected
*Beta**	*bourgaei*	26	7	27	....	....	....
	bourgaei x B. procumbens	49	41	84	49	36	74
	macrocarpa	53	52	98	34	29	85
	maritima	270	211	78	209	146	70
	patellaris	....	....	....	141	75	53
	patula	21	8	38	......	......	......
	procumbens	53	51	96	......	......	......
	scutellaris	28	23	82	......	......	......
	vulgaris var. *abyssinica*	56	49	88	34	23	68
Chenopodium	*album*	....	....	....	32	0	0
	murale	36	0	0	26	0	0

* The seed of the *Beta* species was furnished through the courtesy of Vilmorin-Andrieux and Company, Paris, France. Although originally collected in various parts of Europe the species have been growing in close proximity to each other for several years with every opportunity for cross pollination and therefore cannot be considered pure.

All of the species and varieties of the genus *Beta* which were tested in these trials were found to be susceptible in the seedling stage although there was, in some cases, a marked difference in their response.

Morphologically *Peronospora schachtii* and *P. effusa* resemble each other closely and both infect members of the Chenopodiaceae. In an attempt to compare the pathogenicity of the two fungi, three hosts of *P. schachtii*, Detroit Dark Red beets, White Silver chard, and Giant Lucullus chard; and two hosts of *P. effusa*, Prickly Winter and Long Standing spinach, were sprayed with conidia from *Beta vulgaris* and *Spinacia oleracea*. The results are given in table 3.

In table 3 the percentage of infection in each case was secured by trials, in duplicate, conducted in March 1929. Conidia from *Beta vulgaris* readily infected the variety of garden beets, Detroit Dark Red, and the Swiss chard varieties, White Silver and Giant Lucullus, but produced no infection upon two varieties of Spinach, Prickly Winter and Long Standing. On the other hand, conidia from *Spinacia oleracea* produced no infection on the same varieties of garden beets and Swiss chard while readily infecting the two varieties of spinach. The results show that, as far as the varieties tested are concerned, the host ranges of the two species of *Peronospora* are distinct.

TABLE 3

COMPARISON OF THE HOST RANGE OF PERONOSPORA SCHACHTII AND PERONOSPORA EFFUSA ON VARIETIES OF BETA VULGARIS AND SPINACIA OLERACEA

Host	Variety	Conidia of *P. schachtii* from *Beta vulgaris*			Conidia of *P. effusa* from *Spinacia oleracea*		
		Total plants	Number infected	Per cent infected	Total plants	Number infected	Per cent infected
Beta vulgaris	Detroit Dark Red	150	147	98	135	0	0
Beta vulgaris var. *cicla*	White Silver	122	87	71	147	0	0
B. vulgaris var. *cicla*	Giant Lucullus	108	39	36	73	0	0
Spinacia oleracea	Prickly Winter	130	0	0	117	115	98
S. oleracea	Long Standing	70	0	0	65	58	89

CAUSAL ORGANISM

A description of *Peronospora schachtii* has been given by Fischer,[15] Berlese,[8] and Riehm[36] and will not be repeated here. In the course of these studies certain points have been found which have not been previously presented or which differ from previous descriptions. The coarse, non-septate mycelium can be satisfactorily differentiated from the host tissue in free-hand sections by staining for a few minutes with cotton blue in lacto-phenol. The diameter of the mycelium has been found to vary from 4.4μ to 11.0μ, the average being 6.7μ.

According to Prillieux[34] and Voglino[41], one to three condidiophores emerge from a single stoma. A microscopic observation on young diseased cotyledons has shown in some cases as many as ten conidiophores protruding from a single stoma. From the enlarged

conidiophore base, measuring about 11μ in diameter, the trunk gradually tapers until at the base of the branches the diameter averages approximately 5.7μ. Observations on 200 conidiophores produced in a laboratory moist chamber at 13–15° C have shown that the number of sterigmata on a single conidiophore varied from 5 to 43, the mean being 15 ± 4.4. The total length of the conidiophores varied from 177μ to 653μ, the mean length being $356 \pm 41.4\mu$. The trunk of the conidiophore occupied from two-thirds to three-fourths of the total length, while the branched portions constituted the remainder. These measurements are somewhat larger than those observed by Voglino[41] and Berlese,[8] who stated that the conidiophores were from 250μ to 350μ in length.

Gäumann[19] has shown that the size of conidia in species of *Peronospora* may be greatly influenced by the temperature and humidity during the period of formation and by the stage of maturity of the spore. These results emphasize the importance of making spore measurements under standard conditions which may be duplicated by other workers. Peters[32] apparently made his measurements on fresh conidia while Gäumann used conidia from specimens in exsiccati. In the present studies, measurements have been made on both types of material in order to be able to draw direct comparisons. In the first trial, observations were made on conidia produced on leaves of *Beta vulgaris* in a moist chamber containing a saturated atmosphere at a temperature of 13–15° C. Under these conditions abundant conidia, uniform in size, shape, and general appearance, developed. Germination and infection experiments showed that conidia produced in this manner are functionally mature. Measurements were made with a 4 mm objective and filar micrometer on fresh conidia mounted in tap water. All conidia were disregarded which were obviously immature or which did not present their greater axis at right angles to the line of vision.

In the second trial, conidia which had been dried on a leaf of *Beta vulgaris* in the laboratory for a period of 15 days were used. Following the methods of Gäumann[19], the dried spores were placed in concentrated lactic acid to restore turgidity. The results obtained by measuring 200 fresh and 100 dried conidia are presented in table 4 in comparison with the measurements by Fischer,[15] Voglino,[41] Berlese,[8] Peters,[32] Gäumann,[19] and Riehm.[36] The similarity of the results obtained with fresh and dried spores indicates that the size of conidia is not greatly influenced by the methods used in preparing them for measurement. The writer's measurements correspond

closely to the results of Peters[32] and Fischer,[15] and although the average length of conidia is somewhat less than that recorded by Gäumann[19] and Riehm[36] the differences probably are not significant.

Oospores of *Peronospora schachtii* were first observed in 1882 by Prillieux[33] who reported that they were abundant in the leaves of infected plants. Kühn[24] found no oospores in Germany and at a much later date Peters[32] reported that oospores rarely, if ever, formed in that country.

TABLE 4

MEASUREMENTS OF CONIDIA OF PERONOSPORA SCHACHTII COMPARED WITH MEASUREMENTS OF PREVIOUS WORKERS

Authority	Length in microns		Width in microns	
	Range	Mean	Range	Mean
Fischer[15]	21–26	25	16–21	20
Voglino[41]	20–24	....	15–18	....
Berlese[8]	22–27	...	17–20	....
Peters[32]	....	24	....	20
Gäumann[19]	....	27	....	21
Riehm[36]	....	27	....	21
Present studies, fresh conidia	20.3–28.1	24.0±.96	17.5–24.3	20.2±.81
Present studies, dried conidia	21.9–28.5	24.5±.82	17.5–23.5	19.9±.76

In the San Juan District of San Benito County, California, near the coast, many oospores were found in the infected leaves of beet plants after dessication had occurred. In the Sacramento Valley, however, during the latter part of the growing season when hot, dry weather prevailed, oospores were found only occasionally. The formation of the sexual spore in these cases apparently occurred in connection with the maturity or death of the host tissues. Oospore formation, however, can take place in young, actively growing leaves. Under artificial conditions oospores have been produced in cotyledons and young leaves within 30 days after they were sprayed with a conidial suspension (fig. 10*A*). In such cases the same leaf areas seldom produced both conidiophores and oospores. There is some evidence that low atmospheric moisture inhibits conidiophore production in young seedlings and stimulates the formation of sexual spores. In one young leaf oospores and oogonia were found at the rate of 29,000 per square centimeter of leaf area.

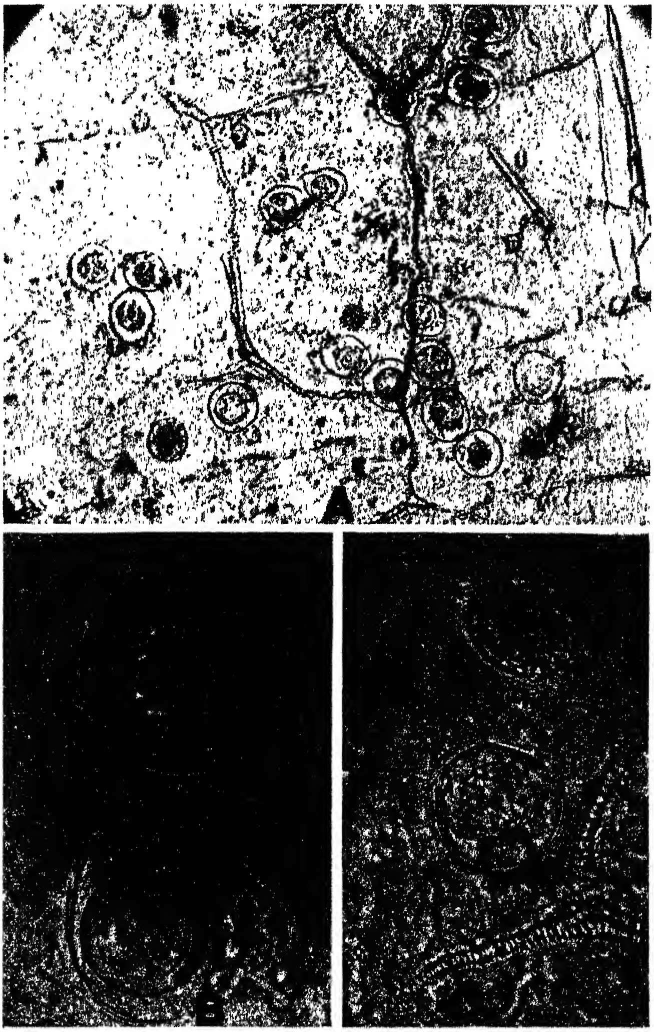

Fig. 10. *A.* Oogonia of *Peronospora schachtii* in beet leaf infected under artificial conditions.

B. Two stages in the development of the oospore; the upper showing the rounding-up of the contents in the center of the oogonium and the lower showing the formation of the oospore wall. An old antheridium remains attached to the oogonium in the latter. x565.

C. Oogonium with antheridium closely appressed to its surface is shown in the center. x330.

The young oogonium of *Peronospora schachtii* appears as a globular, many-nucleated structure surrounded by a thin membrane, the oogonial wall. A single paragynous antheridium usually appresses itself to the periphery of the oogonium (fig. 10*B* and *C*), and the contents of the oogonium round up in the center and become delimited from the periplasm by a definite membrane which thickens to form the oospore wall (fig. 10*B*). Later the periplasm is deposited on the oospore wall as a smooth but irregular epispore, the thin oogonial wall collapsing with the maturity of the oospore. Within the oospore, reserve granules in different states of development have been observed. By measuring 50 apparently mature oospores, it was found that the oospore diameter ranged from 26.6μ to 35.6μ with a mean diameter of 30.8 $\pm$ 1.54μ. It was also found that the oospore wall was from 1.2μ to 3.4μ in thickness with a mean of 2.2 $\pm$ .21μ.

Germination of oospores of comparatively few species of the Peronosporales has been reported. De Bary[6] has shown that the oospores of *Peronospora valerianellae* Fuckel and *Pythium de Baryanum* Hesse germinate by a germ tube while those of *P. proliferum* de Bary and *Phytophthora omnivora* de Bary germinate by a tube ending in a terminal sporangium. De Bary[5] has also shown that oospores of *Cystopus candidus* (Pers.) Lev. germinate by zoospores. The literature on the germination of oospores of *Plasmopara viticola* (Berk. and Curt.) Berl. and de Ton. has recently been compiled by Arens[3]. The germination of oospores of *Pseudoperonospora humuli* (Miyabe and Takah.) Wils. by the production of sporangia which liberate zoospores was shown by Arens.[4] Hiura[20] and Evans and Harrar[14] have shown that oospores of *Sclerospora graminicola* (Sacc.) Schroet. germinate by a germ tube. Peglion[31] on the other hand has shown that the oospores of *S. macrospora* Sacc. produce macroconidia upon short germ tubes. Oospores of *Peronospora spinaciae* (Grev.) Laubert (*P. effusa*) were reported by Eriksson[12] to germinate by a germ tube without passing through a resting period. The only record of the germination of oospores of *P. schachtii* is that reported by Vogliino[41] who stated that oospores placed on a dry leaf of *Beta vulgaris*, germinated by germ tubes, while those placed in drops of water on the leaves germinated by means of zoospores.

During these studies numerous attempts to germinate the oospores of *Peronospora schachtii* have been made without success. This may, in part, be attributed to immaturity of the oospores or to unfavorable conditions of storage. Two methods have been employed in the germination trials. In one, the finely pulverized leaf tissue containing

the oospores, was dusted onto the surface of tap water in watch glasses. The floating cultures were stored at different temperatures between 2° C and 30° C and observations were made at the end of 24 and 48 hours. In the second method, the oospore material was dusted on a piece of filter paper, which was supported on a wire frame above a free water surface and enclosed in a small moist chamber. At the end of 24 and 48 hours, portions of the oospore material were carefully scraped from the filter paper and examined for signs of germination. None was observed in any of the trials.

Conidial Germination.—In early germination trials, conidia were secured from diseased leaves collected out-of-doors early in the morning. In some cases excellent germination was secured by using the conidia removed from such leaves, while at other times not over 50 per cent germination was obtained at the most favorable temperatures. In order to secure more uniform results, heavily infected leaves, collected in the late afternoon, were washed under a strong stream of water while the surface of the leaves was rubbed with the fingers to remove the conidiophores and conidia. The infected leaves were then placed in a moist chamber and held at 13–15° C for 18 to 24 hours, during which time an abundance of fresh conidia developed. A few drops of tap water were placed on the sporulating surface, and repeatedly drawn up into and expelled from a medicine dropper. When the water drops became turbid, due to the presence of large numbers of conidia, the spore suspension was used immediately for the preparation of hanging drop cultures. By using this method uniformly viable conidia, free from foreign material, were secured.

All trials on the relation of temperature to percentage of germination, rate of germination, and rate of germ-tube growth, were made by means of spore suspensions in hanging drop cultures. At first both Van Tieghem cells and hollow-ground slides were used, but after a comparison had shown that there was little or no difference in the percentage of germination by the two methods, only the latter was used.

A simplified technique for making hanging drop cultures with hollow-ground slides was devised. As far as is known, this method is not in use in other laboratories. All of the hollow-ground slides to be used in a series of trials were placed on a laboratory table with the concave surface upward. A glass ring, of approximately the same diameter as the concave area of the slide, was dipped into melted vaseline and quickly touched to each slide in such a manner that a circle of vaseline was formed around the concave area. The required

number of cover slips (25 mm square) were then arranged on the table and a small drop of the spore suspension placed in the center of each. A slide prepared as stated above was then inverted over each cover slip and gently pressed down until the vaseline established contact between the slide and cover slip, forming an air pocket above the drop. The slide and cover slip were then inverted by a quick, continuous motion, without disturbing the drop and the culture was ready for observation or incubation.

Temperature Relation.—For these studies the hanging drop cultures were incubated for 18 to 24 hours in thermostatically controlled chambers, the temperature of which varied less than 1° C. The percentage of germination at each temperature was obtained by counting the spores in four or five fields on each of two or more slides incubated at that temperature. Between 100 and 300 spores were counted on each slide, depending upon the uniformity of germination in the drop.

TABLE 5

EFFECT OF TEMPERATURE* ON THE PERCENTAGE GERMINATION OF CONIDIA OF PERONOSPORA SCHACHTII COLLECTED UNDER FIELD CONDITIONS

Trial	½°	2°	4°	6°–7°	8°	10°	13°	15°–17°	19°	21°	23°–25°	27°–28°	30°
1	....	....	92	82	65	....	11	6	....	6	....	1	....
2	14	21	37	44	38	19	7	6	....	5	....	....	....
3	1	19	44	34	39	27	30	....	6	4	....	....	....
4	5	22	29	45	47	31	24	....	9	8	....	....	....
5	23	82	88	79	....	55	....	3	1	2	1	....	0
6	40	69	83	80	....	75	....	24	10	10	7	....	0
7	30	51	51	62	....	52	....	21	....	6	5	....	0
Maximum per cent germination	40	82	92	82	65	75	30	24	10	10	7	1	0

* Temperature in degrees Centigrade.

The conidia used in all of the trials shown in table 5 were collected out-of-doors in early morning and their lack of uniform maturity accounts for the relatively low percentage of germination at all temperatures in trials 2, 3, 4 and 7. The trials in table 5 were conducted between April 13 and May 4, 1929. The results are not sufficiently uniform to show definitely the optimum temperature, but these studies indicate that 4° C is the most favorable for the germination of conidia of *Peronospora schachtii.* Because of the wide differences in germination at the same temperature, the highest percentage of germination at each temperature represented most accurately the capacity of the conidia for germination.

TABLE 6

EFFECT OF TEMPERATURE* ON THE PERCENTAGE GERMINATION OF CONIDIA OF PERONOSPORA SCHACHTII PRODUCED IN A MOISTURE CHAMBER

Trial	½°	2°	4°	6°-7°	8°	10°	13°	15°-17°	21°	23°-25°	27-° 28°	30°
1	33	93	98	97	....	26	....	3	1	2	....	1
2	....	96	98	98	97	34	....	2	1	1	1	0
3	30	89	97	99	99	61	....	4	1	4	1	....
4	66	74	82	....	87	86	9	....	4	....	1	0
5	4	67	94	....	16	2	1	....	1	6	....	0
6	34	86	96	....	54	32	5	....	1	3	....	1
Total number conidia counted	1099	1873	2085	1020	1600	1943	956	942	2102	2298	1259	2130
Total number germinated	295	1602	1974	999	1147	692	45	25	26	71	11	3
Per cent germination	26.9	85.5	94.5	98.0	71.5	35.6	4.7	2.7	1.2	3.1	0.9	0 1

* Temperature in degrees Centigrade.

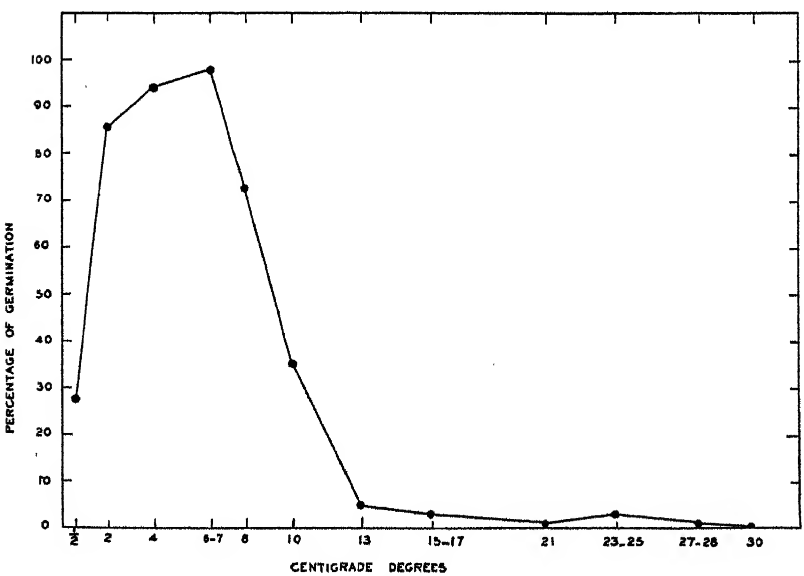

Fig. 11. Relation of temperature to the germination of *Peronospora schachtii* conidia.

The conidia used for the trials reported in table 6, instead of being collected out-of-doors, were produced in a laboratory moist chamber on infected leaves from which the previous conidial coating had been removed by running water. The total number of conidia counted, the total number which germinated, and the percentage of germination for all six of the trials are shown in the summary at the bottom of table 6. The percentage of conidial germination obtained at each temperature from ½° to 30° C is shown graphically in figure 11.

It is evident from the results presented in tables 5 and 6 that, under the conditions of these experiments, the optimum temperature for the conidial germination of *Peronospora schachtii* is from 4° to 7° C. The maximum temperature is approximately 30° C, since only a few trials showed any germination at that temperature. From 4 to 66 per cent germination was obtained at ½° C; therefore, the minimum temperature for conidial germination is apparently near the freezing point.

Although the summary of all the trials shows a higher percentage of germination at 6–7° C than at 4° C, a comparison of the results in trials where the same conidial suspensions were incubated at each of the two temperatures, shows no significant differences. Less than ten per cent germination was obtained in the trials conducted at temperatures above 10° C.

Because of the extreme tolerance of the conidia for low temperature, it was decided to study the effect of freezing on subsequent germination. Duplicate series of hanging drop cultures were prepared and one, the control series, was immediately incubated at temperatures ranging from ½° to 30° C, while the other was frozen at –12° C for 24 hours before being distributed over a similar range of temperatures. The experiment was repeated and the results of both trials showed that conidia were not killed by freezing at –12° C for 24 hours, although the percentage of germination was reduced.

Since conidial germination was not greatly influenced by short periods of freezing, a study of the longevity of conidia held at –12° C was undertaken. A series of hanging drop cultures was prepared and stored at that temperature and at the same time two control cultures were incubated at 2° C to test the viability of the conidia. At intervals of one day or more, duplicate cultures were transferred from the –12° C to the 2° C chamber, where they remained for 24 hours before the percentage of germination was determined. A second series of cultures was later treated in the same manner, except that the slides

were incubated at 4° C after removal from the freezing temperature. The first trial was conducted over a period of 40 days, while the second was extended to cover a period of 100 days.

The results of the trials presented in table 7 show that the percentage of germination is reduced by prolonged freezing. A small amount of germination was observed after the conidia had been stored at –12° C for 40 days, but none occurred after longer periods at the same temperature. From these results it is evident that conidia of *Peronospora schachtii* are not killed by frost and that they could even survive short periods of freezing weather.

TABLE 7

LONGEVITY OF CONIDIA OF PERONOSPORA SCHACHTII FROZEN IN A HANGING DROP AT –12° C

Days at –12° C before germination trials	Per cent germination, trial 1	Per cent germination, trial 2	Days at –12° C before germination trials	Per cent germination, trial 1	Per cent germination, trial 2
1	96	21	10	...	7
2	62	32	15	....	4
3	5	27	20	....	7
4	10	....	25	....	3
5	11	11	30	....	4
6	49	....	40	1	1
7	62	....	50*	....	0
8	0	....	0	91	73
9	0	....	(Control)		

* No conidia germinated after having been frozen for 50 days or more.

The conidia of *Peronospora trifoliorum* de Bary and *Pseudoperonospora humuli* are also able to germinate after short periods of freezing, as has been shown by Melhus and Patel[28] for the former species, and by Arens[4] for the latter.

Infection of host plants by conidia during short periods of favorable conditions depends upon the rapidity of germination and germ tube production. The relation of temperature to the time required for this process was studied by using hanging drop cultures as in previous germination trials. Duplicate cultures were incubated at the same time, and at regular intervals observations were made on the approximate percentage of germination (table 8).

The results presented in table 8 show that, at temperatures from 6° to 25° C, germination was initiated within 2 hours after incubation, while at 4° C, from 3 to 3½ hours were required. At all temperatures the percentage of germination was approximately as great by the end of 4 hours as after 24 hours.

TABLE 8

EFFECT OF TEMPERATURE ON THE TIME REQUIRED FOR CONIDIAL GERMINATION

Temperature, degrees Centigrade	Per cent germination											
	1 hr.	1½ hrs.	2 hrs.	2½ hrs.	3 hrs.	3½ hrs.	4 hrs.	5½ hrs.	7 hrs.	18 hrs.	24 hrs.	48 hrs.
Trial 1:												
4°	0	0	0	0	15	60	90	95	95	95	95	....
6°	0	0	12	50	75	80	95	95	95	95	95	....
8°	0	0	50	75	80	85	95	95	95	95	95	....
Trial 2:												
4°	0	0	0	0	0	40	50	....	60	60	60	60
6°	0	0	0	1	10	20	25	....	25	30	30	30
12°	0	0	3	3	8	9	9	....	10	10	10	10
20°	0	0	1	3	4	4	4	....	4	4	4	6
25°	0	0	1	1	1	2	2	....	2	5	9	28
30°	0	0	0	0	0	0	0	....	0	0	0	0

The relation of temperature to germ tube elongation was determined by measurements made in the same hanging drop cultures which were used for the data presented in table 8. At the specified time intervals the average length of germ tubes in each culture was determined and the results are presented in table 9.

TABLE 9

EFFECT OF TEMPERATURE ON THE TIME REQUIRED FOR GERM TUBE DEVELOPMENT

Temperature, degrees Centigrade	Average length of germ tubes in microns											
	1 hr.	1½ hrs.	2 hrs.	2½ hrs.	3 hrs.	3½ hrs.	4 hrs.	5½ hrs.	7 hrs.	18 hrs.	24 hrs.	48 hrs.
Trial 1:												
4°	0	0	0	0	8	24	32	72	100	240	400	....
6°	0	0	8	16	28	60	94	120	156	320	400	....
8°	0	0	12	24	32	76	104	160	184	320	400	....
Trial 2:												
4°	0	0	0	0	0	3	16	....	60	160	240	320
6°	0	0	0	8	16	20	24	....	80	240	320	400
12°	0	0	16	28	40	62	76	....	170	400	480	580
20°	0	0	24	36	44	56	60	....	150	320	320	340
25°	0	0	48	48	48	48	60	....	120	160	200	320
30°	0	0	0	0	0	0	0	....	0	0	0	0

It is evident that the rate of germ tube growth varied directly with the temperature during the first 3 hours of incubation. However, in trial 1 by the end of 24 hours there was no difference in the average length of germ tubes produced at 4°, 6°, and 8° C. In the second trial

during the first 3 hours the few germ tubes produced at 25° were longer than those produced at lower temperatures. By the end of 24 and 48 hours, however, it was evident that greater germ tube growth occurred at 12°, than above or below this point. Within the first 24 hours germ tubes of 200μ or more were produced at all the temperatures tested, except 30° C.

When conidia on a dry slide were exposed to direct sunlight at 32° to 34° C for 4 hours or more, their power to germinate was destroyed. Those exposed 2 hours or less showed a low percentage of germination after incubation in a drop of water at 4°. Conidia exposed to diffused light indoors at 16° to 25° C for 24 hours or less, did not entirely lose their power to germinate, although the percentage was greatly diminished.

The relation of temperature to the germination of *Peronospora schachtii* conidia was found to be similar to that reported by Hiura(21) for *P. effusa* from *Spinacia oleracea*, except that *Peronospora schachtii* proved to be more tolerant of low temperatures. Hiura found the optimum temperature to be from 8° to 10° C, the minimum below 3°, and the maximum near 30° C. Voglino(41) reported that when conidia of *P. schachtii* were immersed in water or beet decoction for several hours at a temperature of 20–22° C, they germinated by means of zoospores. In the present studies large numbers of conidia have been observed under different conditions and under a range of temperatures from ½° to 30° C, but in no case has a conidium been observed to germinate by means of zoospores.

RELATION OF PARASITE TO HOST TISSUE

Pathogenicity.—To determine if the same conditions which favored conidial germination of *Peronospora schachtii* also favored conidial infection, seedlings of the garden beet variety Detroit Dark Red, growing in steamed soil in four-inch pots, were sprayed with a water suspension of conidia. The inoculum was sprayed on both surfaces of the cotyledons by means of a De Vilbiss atomizer within ten days after the seedlings emerged. Immediately after inoculation the seedlings were incubated for 48 hours at the desired temperature within a chamber in which a nearly saturated atmosphere was maintained by a free water surface. They were then transferred to a greenhouse bench and placed in a chamber kept moist by a layer of wet sphagnum moss. Conidiophores and conidia of *P. schachtii* appeared on the seedlings five to six days after inoculation when the greenhouse

temperature was held between 18° and 24° C. When infected seedlings were stored under similar conditions in a greenhouse held between 8° and 20°, from seven to eight days were required for the development of conidiophores and conidia (table 10). The percentage of infection was determined eight days after the seedlings were inoculated.

TABLE 10

RELATION OF TEMPERATURE TO INFECTION OF BETA VULGARIS SEEDLINGS SPRAYED WITH CONIDIA OF PERONOSPORA SCHACHTII

	Temperature in degrees Centigrade								
	½	2	4	8	10–12	16	20	25	30
Number of trials	4	6	9	8	7	5	7	3	7
Total plants	366	396	728	328	312	631	362	303	333
Number plants infected	314	348	636	296	265	571	282	45	12
Per cent infected	86	88	87	90	85	90	78	15	4

From three to nine trials were conducted at each of nine constant temperatures between ½° and 30° C. The first trial was started on October 15, 1929, and the last on February 10, 1930. The results, summarized in table 10, show that 85 to 90 per cent of the seedlings exposed at temperatures between ½° and 16° C were infected. Seventy-eight per cent of the seedlings were infected at 20°, but at temperatures above that point the percentage was much lower. This information is presented graphically in figure 12. Detailed counts on the percentage of cotyledons and leaves infected were also made but since the results were similar to those for plants they have been omitted from the table. The wide range of temperatures shown to be favorable for infection, as compared to the range favorable for conidial germination, probably may be accounted for by the large quantity of conidia sprayed upon the seedlings.

Judging from the majority of the trials, 8° C is the optimum temperature for conidial infection. The tolerance of this fungus for low temperatures, as indicated in the germination trials, is further shown by the high percentage of infection which occurred at a temperature of ½° C. On one occasion the water in the base of the inoculation chamber was frozen, yet 89 per cent of the plants were infected.

The parts of a beet seedling most susceptible to infection with conidia of *Peronospora schachtii* are the young cotyledons and newly formed leaves. As these structures mature they apparently become

less susceptible. It was found that the cotyledons are susceptible to infection as soon as they emerge from the ground. Whether the relative resistance of leaves of different ages is influenced by the size, number, or distribution of the stomata has not been determined.

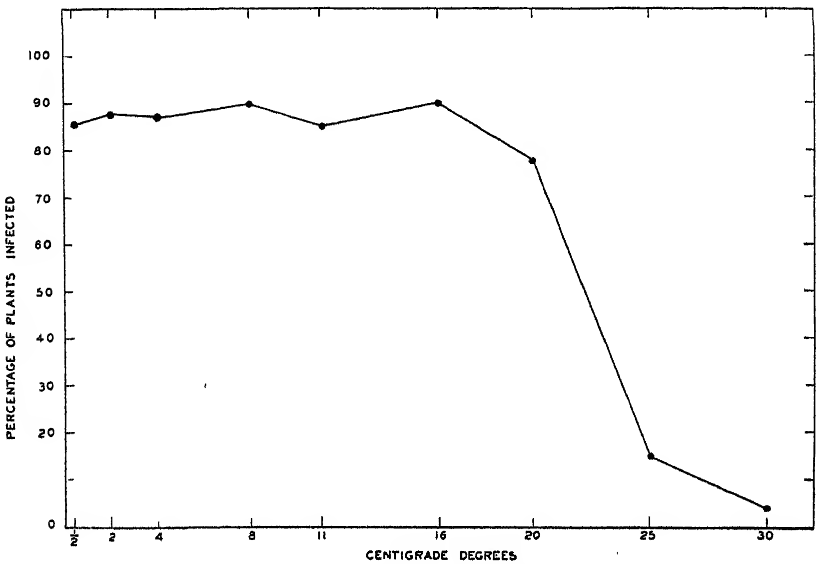

Fig. 12. Relation of temperature to infection of *Beta vulgaris* seedlings by *Peronospora schachtii* conidia.

Mycelial Invasion of the Host.—For observations on the distribution of *Peronospora schachtii* in the tissue of the host, both free-hand and paraffin sections were used. The mycelium and haustoria of *P. schachtii* were, in most cases, readily observed in unstained material, but where the host pigment obscured the fungus mycelium, clearing, by means of 4 per cent potassium hydroxide or concentrated lactic acid, was found advisable. For the study of free-hand sections or macerated tissues, staining with cotton blue in lacto-phenol was found to be more satisfactory than with safranin, although the latter is very useful for quick observations. Magdala red and light green were also used for this purpose. With paraffin sections, Delafield's haemotoxylin and Flemming's triple stain gave the best results. A good differential stain was obtained when sections were left for 48 hours in one part of resorcin blue in 1000 parts of 50 per cent alcohol. Young mycelium and haustoria were stained a brilliant blue, while host material became a dark brown color. The older fungus structures, however, were not so readily distinguished by this stain.

Under field conditions, in the fall of 1929, the first infections were observed in beet root beds six to eight weeks after planting. The fungus was invariably found attacking isolated areas on the older and upper leaves. Attempts to discover a mycelial connection between such infections and the beet crown were made without success. During November, the isolated lesions on the older leaves disappeared or became less conspicuous and the disease appeared abundantly on the young rosette leaves. The new leaves at the center of the rosette were either permeated throughout by the fungus or attacked only along the petiole and base of the blade. Microscopic observations established the fact that there is a continuous mycelial connection between the young infected rosette leaves and the crown of the beet.

It has not been definitely ascertained through what tissues the mycelium gained entrance to the crown, but it seems probable that the infection originated from conidia germinating on the young rosette leaves, rather than from mycelium growing down the petiole from the isolated, diseased areas on the older leaves. Within the crown and neck of the beet, mycelium and haustoria were observed in abundance among all the parenchyma tissues except the pith. In a large number of cases the fungus hyphae were found concentrated near the vascular bundles.

Microscopic studies have shown that mycelium of this fungus may be found throughout the full length of inflorescence axes produced on diseased plants, as was previously reported by Salmon and Ware.[38] The first cauline leaves formed on the floral axis often remain healthy, but the flower shoots produced in the axils of such leaves are usually severely infected. The growth of the fungus keeps pace with the flower development and the mycelium and haustoria can be observed permeating the sepals, pericarp, and filaments, prior to anthesis. Because of the mycelial connection between infected shoots and the crown of the beet through the axis, it is probable that in many cases the infection occurs from the interior of the plant in a manner resembling systemic invasion, rather than from conidial infection on the surface of the flower parts. Following periods of rainfall, however, localized downy mildew infections have often been observed upon the flower parts of otherwise healthy plants. Conidiophores and conidia usually do not appear on the surface of floral parts of *Beta vulgaris* except during cool damp weather. The identity of the fungus mycelium may be definitely established by placing infected floral parts in a moist chamber for a few hours and thus inducing sporulation.

The presence of conidiophores and conidia of *Peronospora arenariae* (Berk.) Tulasne, *P. radii* de Bary, and *P. violacea* Berk. on

the flower parts of host plants has been reported by Fischer.[15] According to Lind,[26] *P. stigmaticola* Raunkiaer occurs only on the flowers of *Mentha aquatica* L. *Peronospora schachtii* was observed on the inflorescence of *Beta vulgaris* by Hollrung[22] and Salmon and Ware[38] but they did not state what tissues were invaded. Recently Cook[11] has shown that mycelium of *Peronospora schleideni* Unger invades the flowers of *Allium cepa* L. and establishes itself within the ovule.[4].

By the use of stained paraffin sections, it has been determined that the mycelium of *Peronospora schachtii* not only permeates the pericarp and sepals of the developing seed ball, but actually invades the integuments of the young ovule. The path of entrance from the pericarp to the integument has not been definitely determined but in some cases mycelial strands were found throughout the full length of the funiculus and oospores were observed near both ends of this structure (fig. 13*C*). The evidence, therefore, points to the funiculus as a possible avenue of entrance into the ovule for the fungus. In many sections of immature seed balls abundant oospores have been observed in the sepals and in the pericarp (fig. 13*A* and *B*).

Material in all stages of development has not been available and, therefore, the relation of the mycelium and oospores in the integument to the developing embryo has not been determined. Penetration of the mycelium into the nucellus and embryo has not been observed. Evidence secured thus far indicates that the fungus mycelium and oospores become imbedded in the seed coat of the mature seed ball. The latter conclusion has been partially verified by dissecting out apparently viable seed from seed balls bearing old conidiophores on their surface. When the seed coat was removed and its inner wall examined microscopically, abundant non-septate mycelium connected to haustoria characteristic of *Peronospora schachtii* was found ramifying among the cells. Similar portions of the seed coats from disease-free seed were entirely free from fungus mycelium.

Although it has not been possible to secure definite proof that the mycelium found within the seed coat is that of *Peronospora schachtii*, the fact that *P. schachtii* conidiophores were found on the surface of the seed balls, that haustoria resembling those of this fungus were

[4] In a recent publication Melhus described and illustrated the presence of mycelium and oospores in the seeds of plants attacked by three species of Peronosporales, *Peronospora alsinearum* Casp. on *Cerastium viscosum* L., *Peronospora viciae* Berk. on *Pisum sativum* L. and *Cystopus bliti* (Biv.) Lév. on *Amaranthus retroflexus* L. (Melhus, I. E. The presence of mycelium and oospores of certain downy mildews in the seeds of their hosts. Iowa State College Jour. Sci. 5:185–188. 1931.)

connected to the mycelium, and that no fungus mycelium was found in apparently disease-free beet seed, makes this conclusion seem probable.

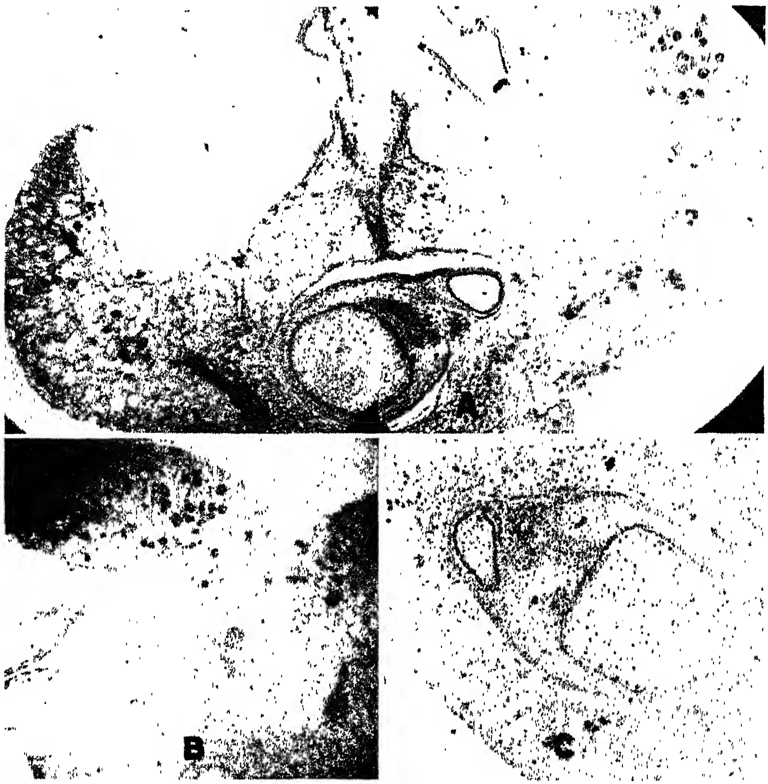

Fig. 13. Oospores of *Peronospora schachtii* in beet seed balls.
A. Longitudinal section of immature seed ball showing oogonia and oospores in the sepals. x44.
B. Oospore production in a sepal. x42.
C. Oospores in the integument of the ovule near the funiculus. x53.

PERENNIAL MYCELIUM OF THE PATHOGENE

Overwintering of several species of the Peronosporaceae by means of perennial mycelium has been established. Melhus[27] compiled the results of his own investigations and those of previous workers, showing that three species of *Phytophthora*, two of *Plasmopara*, one of *Cystopus*, and nine species of *Peronospora* can survive as perennial mycelium.

In addition, Lind[26] states that *Peronospora candida* Fuckel is perennial in the subterranean parts of *Primula elatior* Hill. By means of extensive investigations Murphy,[29] and Murphy and McKay,[30] have shown that *Peronospora schleideni* hibernates as perennial mycelium, while Salmon and Ware[37] and Ware[42] have, in the same way, shown that the mycelium of *Pseudoperonospora humuli* is perennial. Gardner[18] presented evidence that *Peronospora parasitica* (Pers.) de Bary, previously shown by Melhus[27] to survive on *Lepidium virginicum* L., could hibernate in the roots of the turnip (*Brassica rapa* L.). Klebahn[23] has reported that *Peronospora pulveracea* Fuckel lives over as perennial mycelium in the rootstocks of *Helleborus* sp.

Kühn[24] stated that he had proved by repeated experiments that *Peronospora schachtii* can survive the winter as mycelium in the crowns of beets stored for seed production, and reappear on the young leaves when the beets are planted the following spring.

To test the hibernation of the fungus in the crown of stored stecklings, 79 diseased and 18 healthy beets were collected in July, 1929, and stored in sawdust at 4° C for 100 days. The beets were then planted in steamed soil in the greenhouse. Twelve of the healthy plants survived the storage period and none showed any signs of *Peronospora schachtii* on the new leaves. Only 3 of the 79 diseased plants survived and one of these produced leaves bearing typical conidiophores and conidia. The low percentage of plants which survived probably indicates that the conditions of storage were not ideal; but at the same time, a comparison with the healthy plants clearly shows that *P. schachtii* weakened the infected plants and provided a means of entry for saprophytic fungi and bacteria. The one case of successful hibernation, however, indicated that the conclusion of Kühn[24] on this point was justified.

In regions having severe winters the perennial mycelium in stecklings stored for seed production serves as a means of overwintering. In central California the root beds are planted in the early fall, the beet roots or stecklings are transplanted direct to seed fields in December or January, and the entire life cycle of the plant is completed in a ten-month period of continuous growth. Mycelium of *Peronospora schachtii* often invades the crown of beet seedlings while they are still in the root beds and when such plants are moved to the seed fields they initiate centers of infection. The perennial mycelium, in this case, serves to carry the fungus from the root beds to the seed beet fields.

In the same way, the perennial mycelium can carry the fungus through the summer period when weather conditions are unfavorable and when no commercial beet crop is growing in the region devoted to seed production. This was demonstrated in the summer of 1929 on a number of diseased beets which were being maintained in an experimental plot at Davis, California. When extremely hot dry weather occurred, all external symptoms of the disease disappeared from the plants, although microscopic examinations showed that the fungus mycelium was still abundant in the crown of many of the beets. Several months later when the weather was cooler, the conidiophores and conidia of *Peronospora schachtii* appeared on the newly formed leaves. Since there were no other beet plants in the vicinity, this observation serves as a verification of the fact that *P. schachtii* does survive unfavorable periods as mycelium within the beet crown.

The discovery by Prillieux[33] of abundant oospore production by this fungus in beet leaves under certain conditions, provides a second source from which beet plantings may become infected.

Although Prillieux,[33] and Salmon and Ware[38] have suggested the possibility of *Peronospora schachtii* being perpetuated by oospores in the soil, no reference to controlled experiments substantiating this suggestion has been found. Trials have been conducted to secure information as to the possibility of *P. schachtii* being carried over by oospores in the soil. Soil was collected from a seed field and a market garden, both of which had produced diseased crops during the previous season. *Beta vulgaris* seed from a disease-free region was planted in pots filled with the soil collected, part of each lot of soil having been previously steam sterilized for use as a control. Seven hundred and eight beet seedlings developed in 67 pots of field soil, and 153 developed in the 24 pots of sterilized soil. No infection occurred in any of the pots. Because of the meager information available concerning the conditions necessary for oospore germination and infection, the negative data presented above cannot be considered significant.

When seed of *Beta vulgaris* was planted in steam sterilized soil in which had been mixed abundant pulverized leaf material containing oospores of *Peronospora schachtii*, only one seedling in each of two trials became infected. Three other trials gave negative results and no infected seedlings were observed in any of the control pots.

SEED TRANSMISSION

Eriksson[13] in 1924 observed a small number of infected sugar beet plants scattered throughout an otherwise disease-free field and concluded that this condition was probably the result of seed transmission. This hypothesis was given credence by the fact that Hollrung[22] described the presence of *Peronospora schachtii* on the inflorescence of seed beets. The latter author, however, stated that there was no proof that the disease could be transferred through the seed.

Clinton[10] has shown that the mycelium and oospores of *Phytophthora phasaeoli* Thaxt. are present in the seed of lima beans (*Phaseolus lunatus* L.). He found, however, that infected seed which germinated did not produce diseased seedlings and concluded that the primary infection of young seedlings originated not directly from mycelium in the cotyledons, but from zoospores produced by the mycelium or oospores carried over in the seed.

From the results of controlled seed germination trials Angell[1] tentatively concluded that "blue mould" of tobacco (*Peronospora* sp.) can be transmitted by seed from severely infested fields. Cook[11] recently reported the presence of mycelium of *Peronospora schleideni* in the floral parts of *Allium cepa* L. and suggested the possibility of seed transmission.

It has been shown that the mycelium of *Peronospora schachtii* may invade the seed ball and establish itself in the pericarp and inner layers of the seed coat either with or without the formation of oospores (fig. 13*A*, *B* and *C*). The invasion of the nucellus, endosperm, or embryo has not been observed. There is also the possibility that oospores produced elsewhere may become lodged in the crevices of the seed ball and serve as a source of infection to the young seedlings. Unfortunately no method of growing *Peronospora* species in pure culture has yet been devised. Therefore, to determine the viability of the mycelium or oospores within the seed balls, it was necessary to germinate the seed under controlled conditions and observe the presence or absence of seedling infection.

In order to conduct experiments on the seed transmission of this fungus it was necessary to secure a supply of seed known to have been infected, or exposed to infection. A large number of infected flower shoots were tagged soon after pollination. Examination of these shoots at a later date revealed the fact that, almost without

exception, flowers which were infected in an early stage of development failed to mature. Many of the seed balls which developed were so badly shriveled that they could be readily removed by ordinary commercial cleaning practices. Less severely infected flowers did, however, produce viable seed. The material finally selected for the transmission trials consisted of: first, seed from infected plants, second, random selected seed from infested fields, third, commercial seed from diseased fields, and fourth, commercial seed, part of which had been used for planting fields that were found to contain infected plants. Commercial seed from the Pacific Northwest, a region in which *Peronospora schachtii* has never been reported, was used for the controls. The soil used in all trials and the containers were steam sterilized at 40 pounds pressure for two hours. After the seedlings emerged, the flats and pots containing them were enclosed by sterilized cloth covers which were removed only for watering and observation. In the early trials the seedlings were watered with a fine spray to increase the humidity, but in later trials the water was poured down the side of the container to avoid splashing any material on the seedlings.

In the first trial, 14 samples of seed from individual diseased plants were planted on November 15, 1929, and within 60 days one or more infected seedlings appeared in ten of the samples. In addition, one infected seedling was found among 400 produced from random selected seed, while another such seed sample produced none. Diseased seedlings were also found in one of the two lots produced by commercial seed harvested from infested fields and from one of the two lots of seed which had previously produced field plantings of diseased beets. European grown sugar beet seed, which had produced a field planting containing one per cent of infected plants in the spring of 1929, produced three infected seedlings out of 1000.

The second series of trials, started on January 20, 1930, was similar to the first, but on a much larger scale. Two weeks before starting the trials, the benches, walls, and floor of the greenhouse were washed down carefully and all susceptible host material was removed. Steamed soil and containers were used as before and new cloth covers were made.

The temperature was somewhat higher than during the previous trials and apparently more favorable for seedling development, since a shorter period was required for emergence, and subsequent growth was more rapid. That the fungus development was also influenced is shown by the fact that the incubation period from planting to the

appearance of infection was, in this case, from 24 to 33 days, while in the earlier trials from 45 to 60 days had been required.

Out of 21 seed samples from single diseased plants 9 produced infected seedlings, of which 5 had produced diseased seedlings in the former trials. Infected seedlings were also found in three lots of commercial seed harvested from infested fields and in two lots of seeds which had produced fields of infected plants. No infection was observed in five lots of control seedlings.

TABLE 11

RESULTS OF TESTS ON TRANSMISSION OF PERONOSPORA SCHACHTII THROUGH SEED OF BETA VULGARIS COLLECTED FROM INDIVIDUAL DISEASED PLANTS

Sample number	Number trials	Number seedlings	Number seedlings showing primary infection	Per cent primary infection
1	1	200	0	0.00
2	2	1,450	3	0.20
3	2	520	1	0 20
4	2	110	1	0.91
5	2	260	1	0 38
6	2	1,020	6	0 59
7	3	1,720	6	0 35
8	2	740	0	0.00
9	3	1,680	5	0.30
12	1	150	1	0.67
13	3	3,040	10	0.33
14	1	200	1	0.50
16	1	50	0	0.00
20	2	820	1	0.12
23	1	360	0	0.00
24	1	350	1	0.29
25	1	45	0	0.00
26	1	275	0	0.00
29	1	100	0	0.00
30	1	100	0	0.00
35	1	161	0	0.00
36	2	540	1	0.19
40	1	975	1	0.10
15*	1	150	0	0.00
19*	1	400	1	0.25
39*	1	290	0	0.00
Totals for above trials..................	40	15,806	40	0.25

* Composite sample collected from several diseased plants.

The results of all the trials are summarized in tables 11 and 12. Twenty-three seed samples collected from individual diseased plants were planted under isolated conditions, of which 14 have produced infected seedlings in one or more trials. In some cases the same seed

sample produced a few infected seedlings in one trial but none in the second trial. On the other hand, certain samples, such as numbers 7, 9, and 13, produced diseased seedlings in each of the three trials. Irregularities in the results may be accounted for by the apparently small percentage of primary infection. In these studies only the first infected seedlings observed in each container and those showing infection before the elapse of five days, the conidial generation period, were considered to represent primary infections.

TABLE 12

RESULTS OF TESTS ON TRANSMISSION OF PERONOSPORA SCHACHTII THROUGH COMMERCIAL SEED OF GARDEN BEETS, SUGAR BEETS, AND MANGELS

Sample number	Number trials	Number seedlings	Number seedlings showing primary infection	Per cent primary infection
Garden beets				
10	2	1,970	3	0.15
17	3	2,010	5	0.25
21	2	940	1	0.11
22	2	725	1	0.14
61	1	190	0	0.00
62	1	145	0	0.00
63	3	885	0	0.00
64	2	780	0	0.00
65	2	286	0	0.00
66	1	150	0	0.00
69	1	440	0	0.00
70	1	610	0	0.00
71	1	450	1	0.22
72	1	490	1	0.20
73	1	420	0	0.00
74	3	3,490	7	0.20
Sugar beets				
18	4	4,820	8	0.17
Mangels				
38	1	500	0	0.00
Totals for above trials	32	19,301	27	0.14

Results obtained with seed samples 10 and 17, harvested from severely infested commercial seed beet fields, show that not all infected seed balls are removed in cleaning. A few infected seedlings were produced in each planting made from this material. Seed samples 61, 62, and 63 from the Pacific Northwest where *Peronospora schachtii* is as yet unknown, produced no diseased seedlings under similar conditions.

In some cases it has been possible to correlate field observations with greenhouse experiments. For example, seed sample 21, secured through an Eastern seed company but the original source of which is unknown, was used to plant two commercial fields in 1929, both of which later developed infected plants. In one case the beet planting was well isolated, while in the other case it was near another beet planting which remained apparently free of the disease. In the greenhouse trials this seed sample produced a few infected seedlings. Seed sample 18 was used to plant a large commercial sugar beet field in the spring of 1929. In May a count was made in this field and approximately one per cent of the plants, uniformly distributed throughout the planting, were found to be infected. In the greenhouse among 4820 seedlings, 8 primary infections were observed.

The percentage of seedlings which are infected by fungus material carried with the seed ball can only be approximated. From these studies, however, it appears that seed from plants known to be diseased seldom produces more than one per cent of infected seedlings. From the eight samples of commercial seed found to be infected, only 0.18 per cent of the seedlings were diseased. No attempt has been made as yet to determine whether the inoculum carried with the seed ball exists as mycelium or oospores.

Throughout these trials every precaution was taken to prevent outside infection, and since certain samples of seed repeatedly produced infected seedlings, while others always produced seedlings free of infection, the conclusion that *Peronospora schachtii* is transmitted by means of the seed seems justified.

CONTROL

During the progress of these investigations no experimental evidence on the control of *Peronospora schachtii* has been obtained. The information secured points to the advisability of certain practices which should, theoretically, reduce the losses suffered from this disease. Downy mildew of beets is apparently carried with the seed and, therefore, plantings should be made with seed from disease-free sources. The abundant production of oospores under certain conditions no doubt produces soil infestation. No evidence is available as to the longevity of the oospores in the soil, but it is advisable to avoid, wherever possible, planting beets in fields where the disease has previously occurred.

In root beds the presence of downy mildew symptoms on the youngest rosette leaves is usually associated with mycelium in the crown. It therefore seems advisable, when transferring beet plants from the root beds to the seed-beet fields, to eliminate all plants showing this type of infection.

Spraying with Bordeaux mixture and dusting with copper-lime dust are now being practiced by certain growers in an attempt to check the disease in the root beds and seed fields. Although studies are now in progress, no definite information as to the effectiveness of these treatments is yet available.

SUMMARY

Peronospora schachtii has been known to occur to a limited extent on sugar beets in California since 1911 but it was not until 1929 that the disease became a serious factor in garden beet seed production. In 1929 an estimated loss of 43 per cent was incurred from this disease on 600 acres of garden beets grown for seed in the Sacramento Valley. The disease is serious also on market beets in the coastal trucking sections south of San Francisco.

The fungus attacks plants in all stages of development. Under artificial conditions the disease readily appears on cotyledons and young leaves of seedling plants, resulting in a bleaching and downward curling of the cotyledons, and a distortion of the young leaves. Infected cotyledons and young leaves show a heavy gray coating of conidiophores and conidia on their lower surface.

Under field conditions, the first symptoms of downy mildew in the beet root beds usually occur as isolated irregular lesions on older leaves. After the fall rains begin, the disease is confined chiefly to the youngest leaves, causing the center of the beet rosette to become a mass of small, tightly curled and distorted leaves covered with a mass of conidiophores and conidia. In the seed-beet fields, *Peronospora schachtii* infection on the inner rosette leaves is the most conspicuous symptom of the disease, but during periods of rainfall secondary infection occurs as isolated areas on the outer leaves. Flower shoots produced on diseased plants are often invaded systemically, which results in the entire inflorescence showing a stunted and compact form of growth. Infected bracts and flower parts are swollen, distorted, and faded in color.

Field observations on the development of a downy mildew epidemic in a beet root bed during the fall of 1929 showed almost conclusively that all subsequent infection spread from two primary centers.

Inoculation of young plants, under controlled conditions, has shown the following to be susceptible to the disease: *Beta vulgaris* (ten varieties of garden beets, nine varieties of mangels and three varieties of sugar beets), *B. vulgaris* var. *cicla* (two varieties of Swiss chard), *B. bourgaei*, *B. bourgaei* × *B. procumbens*, *B. macrocarpa*, *B. maritima*, *B. patellaris*, *B. patula*, *B. procumbens*, *B. scutellaris*, and *B. vulgaris* var. *abyssinica*. No infection was obtained on *Chenopodium album* and *C. murale*. Experimental evidence showed that the downy mildew on *Beta vulgaris* and that on *Spinacia oleracea* are pathogenically different.

Controlled temperature studies have shown that a temperature of 2°–10° C is favorable for conidial germination, with an optimum between 4° and 7° C. The minimum is below ½°, while the maximum is near 30° C. Conidial germination was initiated within 2 hours at temperatures from 6° to 25° but at 4°, 3 to 3½ hours were required. The length of germ tube varied from 240μ to 480μ at the end of the first 24 hours with the greatest development occuring at 12° C. Short periods of freezing did not affect the viability of conidia and a few were found to be capable of germinating after 40 days at –12° C.

A high percentage of infection was obtained on beet seedlings sprayed with conidial suspensions at temperatures between ½° and 20° C. Slight infection was obtained at 30° C. Cotyledons and newly formed leaves were found to be the most susceptible portions of the beet seedlings.

Abundant oospores were found in the leaves and floral parts of seed beets and in cotyledons and young leaves of seedlings exposed to infection under artificial conditions. Attempts to germinate the oospores have been unsuccessful. Oospore inoculations in sterile soil resulted in infection on a single beet seedling in each of two trials. No infection was obtained in three other trials.

Microscopical studies of free-hand and paraffin sections have shown that the fungus mycelium spreads from the young infected rosette leaves of the beet into the crown. Leaves and flower shoots produced after the mycelium invades the crown are usually completely invaded by the fungus.

Mycelium and oospores were found abundantly in the pericarp and sepals of beet flowers and occasionally were present in the funiculus and the integuments of the ovule. Mycelium and haustoria re-

sembling those of *Peronospora schachtii* were found inside the testa of viable seed from seed balls bearing dry conidiophores on their surfaces.

Twenty-seven samples of beet seed collected from diseased plants and 18 samples of commercial seed were planted in sterilized soil in the greenhouse. Infected seedlings appeared in 15 lots of the former and in 8 lots of the commercial seed. Primary infection occurred on 0.10 to 0.91 per cent of the seedlings from seed produced on diseased plants and on 0.11 to 0.25 per cent of those from commercial seed. Seed from disease-free regions produced no infected seedlings. It was therefore concluded that *Peronospora schachtii* is transmitted with the seed.

Field observations and greenhouse trials confirmed the conclusion of earlier investigators that *Peronospora schachtii* may hibernate by means of perennial mycelium in the beet crown.

No adequate control measures are as yet known. Life history studies and field observations indicate that the use of disease-free seed, avoidance of infested fields, and elimination of infected stecklings are advisable.

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MODES OF CURLY-TOP TRANSMISSION BY THE BEET LEAFHOPPER, EUTETTIX TENELLUS (BAKER)

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INTRODUCTION

A number of scientists have worked on the incubation period of the curly-top virus in the beet leafhopper, *Eutettix tenellus* (Baker), in which the infective principle passes into the mouth parts, alimentary canal, blood, salivary glands and out of the mouth parts in sufficient quantity to produce infection. It has been known for a long time that previously noninfective beet leafhoppers are able to transmit curly top in short periods after feeding on diseased beets. A number of theories have been published recently attempting to explain these short periods of curly-top transmission. A short review of the literature on this subject is given in the following paragraphs.

Smith and Boncquet[13] state that not more than 3 hours of feeding were necessary to obtain the pathogenic factor, but that a period of at least 24 hours and not more than 48 hours must elapse before the insect could transmit the disease. These facts suggested to them that curly top was not transferred mechanically, but that some development or change took place within the body of the insect during the first few hours after feeding on a diseased plant.

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Severin[7] found that adults previously noninfective were occasionally able to transmit curly top within 4, 5, or 6 hours after feeding on a diseased beet under high temperatures (mean 100.0° to 115.6° F). Eight cases (13.3 per cent) of curly top developed from 60 beets used in this experiment. Lots of 25 or 50 males were used instead of single leafhoppers. Curly top was not produced by beet leafhoppers with hourly trials of from 1 to 10 hours under lower temperatures (maximum 80°–86° F) in the greenhouse during October and November.

Severin[9] also reported that 100 lots of 25 or 50 adults failed to transmit the infective principle of curly top to 114 beets within 1, 2, or 3 hours. In two experiments, 9 lots of from 100 to 1,000 nymphs or adults failed to communicate the virus to 27 beets within 2, 3, and 4 hours. Beet leafhoppers previously noninfective transmitted curly top to 15 of 126 beets (11.9 per cent) within 4, 5, 6, and 10 hours. He found later[11] that leafhoppers previously noninfective transmitted curly top within 2 hours after feeding on a diseased beet.

Later experiments included in this paper showed shorter periods of curly-top transmission by the beet leafhoppers.

Swezy[14] found two large masses of bacteria in the lumen of the esophagus anterior to the esophageal valve (fig. 1) in a single beet leafhopper of 250 specimens examined. Similar masses of bacteria were found in the midintestine of other specimens, but the esophageal valve was not clogged. She assumed that the two masses of bacteria anterior to the valve might hinder the free passage of food into the midintestine and that it might be possible for the infected beet juice to be regurgitated from the esophagus through the mouth parts. She concluded that "if, as seems very probable, this is the case, this condition would explain the occurrence of infection in ½ or 1 hour."[14]

Bacot and Martin[1] found that two species of rat fleas, *Xenopsylla cheopis* and *Ceratophyllus fasciatus,* can transmit *Bacillus pestis,* the cause of bubonic plague, through regurgitation from the foreintestine. When the alimentary canals of fleas showing successive stages of development of the bacilli are examined (fig. 2), it is evident that the proventricular valve is clogged with bacteria and extends into the esophagus and stomach. When fleas in this condition feed, the contaminated esophagus becomes distended with blood, and when the pharynx ceases to pump, some of the blood is regurgitated from the esophagus and is forced out of the mouth parts. Such fleas are persistent in their endeavor to feed, and this renders them particularly dangerous.

Swezy[14] fed beet leafhoppers on stains dissolved in a sugar solution and found that the cells of the midintestine were stained in 15 minutes and the salivary glands at the end of 1 hour under high temperatures. It was assumed from this experiment that an infective organism might remain unchanged in its passage through the midintestine, blood, and salivary glands and when injected with the saliva into a healthy beet seedling might occasionally produce infection at short intervals.

Fig. 1. Photomicrograph of esophageal valve with two masses of bacteria lodged in the esophagus anterior to it. (After Swezy.)

Another theory suggested by Swezy[14] is as follows: "An alternative explanation is found in the fact that a change in the life cycle of the infective organism occurs in the body of the insect and this must be completed before the insect is capable of readily transmitting the disease to a healthy plant. This explanation is not incompatible with the finding of an occasional transmission of curly top within such intervals as 1 or 2 hours."

In this paper further experiments are reported on short periods required for previously noninfective insects to transmit curly top to healthy beets after feeding on diseased beets. Investigations were also undertaken on the transmission of curly top by single beet leaf-

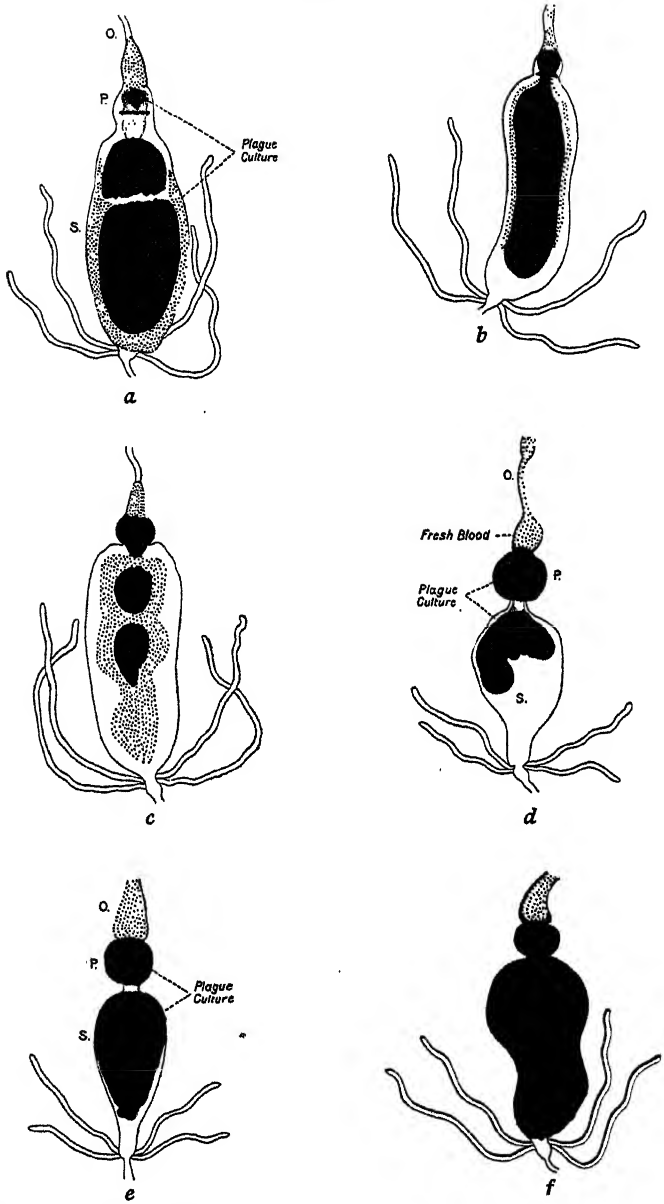

Fig. 2. Alimentary canals of fleas showing successive stages of development of *Bacillus pestis*, the cause of bubonic plague. The bacilli grow so abundantly as to clog the proventriculus. Fleas in this condition are not prevented from sucking blood, but when the pharynx ceases to pump, regurgitation of the contaminated blood occurs through the esophagus and some of the blood is forced out of the mouth parts. Plague cultures are shown black; fresh blood, stippled. *O*, esophagus; *P*, proventriculus; *S*, stomach. (Adapted from Bacot and Martin.)

hoppers in longer periods, the relation of mass inoculation by groups of insects to curly-top transmission, and the virus incubation period in the insect. Experiments were conducted on curly-top transmission by contamination of mouth parts. Inoculation experiments were also undertaken with the feces of infective beet leafhoppers.

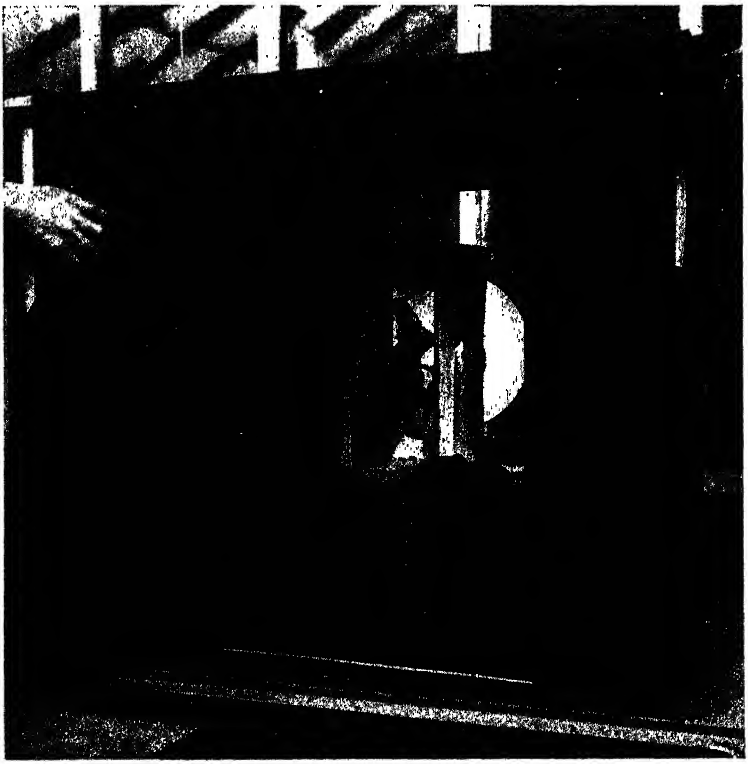

Fig. 3. Dark chamber in which transfers of beet leafhoppers were made to prevent escape of adults. If a leafhopper escaped, it was attracted to the light of an electric lamp enclosed in a reflector outside of the glass plate.

METHODS

The methods used in the transmission of curly top by the beet leafhopper varied in different experiments. Beet leafhoppers were either fasted overnight or during the morning in a cool room in many experiments. The insects, within cages, were fed on curly-top beets from which the outer leaves not showing symptoms of the disease had been removed. The hoppers were transferred to healthy beet seedlings

with from 6 to 12 leaves. The removal of the cages from the beets was performed in a dark chamber provided with a glass plate, outside of which was a 50-watt electric lamp covered with a shade (fig. 3), so that any adults that remained on the plant, resting between the petioles, were attracted to the light after the cage was removed. Precautions were taken that all of the insects were removed from the plants in the transfers. The inoculated beets were fumigated with Nico-Fume tobacco-paper insecticide and were kept within insect-proof cages for a period of 3 months if symptoms did not develop within the usual period of 1 to 2 weeks.

SHORT PERIODS OF CURLY-TOP TRANSMISSION BY BEET LEAFHOPPERS

In determining the short periods of curly-top transmission by the beet leafhopper, high temperatures were usually used, so that the insects would feed during the short periods on the diseased and healthy beets. In most experiments the hoppers were transferred to successive healthy beet seedlings. After the last transfer of the insects the hoppers were counted. The length of time that the leafhoppers were left on diseased and healthy beets is given in table 1. The number of leafhoppers used is given only when infection was produced.

It is evident from table 1 that curly top was transmitted to a healthy beet seedling within ⅓, ½, 1, 1½, 2, 3, and 4 hours. Table 1 shows that the percentage of curly-top transmission varied from 2.4 per cent within ⅓ hour to 33.3 per cent within 4 hours.

It is evident from table 1, that each lot of 178 and 350 hoppers caused infection in 3 successive beets. On the other hand, a lot of 1,000 insects transmitted the infective principle within 2 and 4 hours, but failed in the 3-hour test. A lot of 1,153 leafhoppers transmitted curly top within 2 and 3 hours but failed in 4 hours.

When 20 to 50 adults were used, the transmission of curly top within ⅓, ½, 1, 1½, 2, 3, and 4 hours averaged 7.3 per cent with 96 beets, as compared with 22.5 per cent with 129 beets when more than 50 hoppers were used. No infections were obtained when 5 to 15 insects were used.

Trials conducted in late summer or in the autumn were not as successful as during the months of May, June, and July, in which high temperatures can often be obtained in the fog belt at Berkeley.

TABLE 1

SHORT PERIODS OF CURLY-TOP TRANSMISSION BY BEET LEAFHOPPERS

Total period on curly-top and healthy beets, hours	Length of time, hours		Number of beets infected	Number of beets inoculated	Per cent infected	Number of leafhoppers used on beets that developed curly top	Temperatures during feeding period of leafhoppers, degrees F		
	On curly-top beet	On healthy beet					Maximum	Minimum	Mean
1/3	1/6	1/6	1	41	2.4	40	110	102	106.0
1/2	1/4	1/4	1	14	14.3	40	109	104	106.5
1/2	1/4	1/4	1			300	110	105	107.5
1	1/2	1/2	1	34	2.9	50	110	104	107.0
1 1/2	1	1/2	1			20	96	94	95.0
1 1/2	1/2	1	1	24	16.7	40	96	94	95.0
1 1/2	1/2	1	1			95	125	114	119.5
1 1/2	1/2	1	1			127	112	102	107.0
2	1/2	1 1/2	1			34	129	114	121.5
2	1	1*	1			178	116	102	109.0
2	1	1*	1	40	15.0	350	116	102	109.0
2	1	1*	1			1,000	116	102	109.0
2	1	1*	1			1,071	116	102	109.0
2	1	1*	1			1,153	129	114	121.5
3	1/2	1	1			25	132	114	123.0
3	1	1*	1			178	116	102	109.0
3	1	1*	1			332	116	102	109.0
3	1	1*	1			350	116	102	109.0
3	1	1*	1	36	27.8	455	116	102	109.0
3	1/2	1	1			515	118	102	110.0
3	1	1*	1			578	116	102	109.0
3	1	1*	1			864	116	102	109.0
3	1	1*	1			1,071	116	102	109.0
3	1	1*	1			1,153	116	102	109.0
4	1/2	1	1			80	133	114	123.5
4	1/2	1	1			95	133	114	123.5
4	1/2	1	1			127	120	102	111.0
4	1	1*	1			178	116	102	109.0
4	1/2	1	1			229	120	102	111.0
4	1	1*	1	36	33.3	291	113	106	109.5
4	1	1*	1			332	116	102	109.0
4	1	1*	1			350	116	102	109.0
4	1	1*	1			448	116	102	109.0
4	1	1*	1			864	116	102	109.0
4	1	1*	1			1,000	116	102	109.0
4	1	1*	1			1,071	116	102	109.0

* Fed 1 hour each on successive healthy beets.

Curly top was not transmitted to any of 42 beets within 2, 3, or 4 hours after the hoppers had fed on diseased beets on August 21 and September 6. The number of hoppers varied from 133 to 405 on each beet, a total of 3,759 being used in two experiments. The insects were

fed for a period of 1 hour on the diseased beets and were then transferred to successive healthy beets. At the end of each test each lot of leafhoppers was kept on a healthy beet for 2 weeks to determine whether they became infective in feeding for a period of 1 hour on a diseased beet. Fourteen lots of hoppers used in the two experiments transmitted curly top to 14 beets.

It has frequently been observed that recently molted adults, after the chitin has hardened, feed for several hours. It was, therefore, decided to test these insects for short periods of transmission of the virus. Twenty previously noninfective male leafhoppers after passing through the last molt were kept in a cage without food for a period of 3 to 4 hours, and then each specimen was fed on a different diseased beet for an hour. Each insect was disturbed in its meal and fed on a separate healthy beet for another hour, but not a single case of curly top developed.

TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS

Experiments conducted up to the present time using one insect in each test seem to demonstrate that a single beet leafhopper does not transmit curly top in short periods. As stated in a previous paper[7] 44 previously noninfective nymphs were fed singly upon diseased leaves for a period of 1 to 2 minutes, and each nymph was then fed on a healthy beet for 5 minutes, or less, if it completed its meal before the end of that time, but not a single case of curly top developed. It was demonstrated, however, that 21 previously noninfective nymphs fed from 1 to 2 minutes on a diseased beet, and then fed on a healthy beet for a period of two weeks, transmitted curly top.

In a later experiment 20 nymphs were fed singly upon diseased beets for a period of 1 to 4 minutes and then each hopper was fed on a healthy beet from 3 to 22 minutes until it completed its meal. In another experiment 14 nymphs and 6 adults were fed singly upon curly-top beets for 10 minutes and then each hopper was disturbed in its meal and transferred to a healthy beet seedling for 10 minutes. Negative results were obtained with all of the 40 beets.

In the next experiment previously noninfective beet leafhoppers were fed singly on diseased and healthy beet seedlings for periods varying from 2 to 11 hours under high temperatures, as indicated in table 2. Each leafhopper was fed on a diseased beet seedling showing either the early symptom of the disease, namely, the transparent

venation, or the wart-like protuberances on the lower surface of the leaves. The outer leaves without symptoms were removed from each beet. A similar experiment was also undertaken with infective male beet leafhoppers which had completed the nymphal stages on a diseased beet. Each male was left on a healthy beet seedling for periods varying from 1 to 11 hours. The results are shown in table 2.

TABLE 2

TRANSMISSION OF CURLY-TOP VIRUS BY SINGLE BEET LEAFHOPPERS

Number of leafhoppers kept singly on each beet	Length of time, hours		Number of beets inoculated	Number of beets infected	Number of beets healthy	Per cent of beets infected	Temperatures during time leafhoppers were left on curly-top and healthy beets, °F		
	On curly-top beet	On healthy beet					Maxi-mum	Mini-mum	Mean
Previously noninfective leafhoppers									
10	1	1	10	0	10	0	116	110	113.0
10	1	2	10	0	10	0	100	92	96.0
15	2	1	15	0	15	0	107	100	103.5
10	2	2	10	0	10	0	100	82	91 0
15	3	1	15	0	15	0	116	92	104.0
10	4	1	10	0	10	0	102	92	97.0
13	3	3	13	0	13	0	100	92	96.0
15	5	1	15	0	15	0	92	82	87.0
15	6	1	15	1	14	6.7	100	88	94.0
10	4	4	10	0	10	0	100	82	91.0
14	7	1	14	0	14	0	98	71	84.5
16	8	1	16	1	15	6.2	102	82	92.0
14	9	1	14	0	14	0	100	80	90.0
16	5	5	16	1	15	6.2	102	82	92.0
10	3	8	10	1	9	10.0	100	82	91.0
10	10	1	10	0	10	0	100	80	90.0
Total	......	......	203	4	199	2 0	......	......	......
Infective leafhoppers									
20	Reared on curly-top beets	1	20	5	15	25 0	104	95	99.5
20		2	20	2	18	10.0	104	98	101.0
12		3	12	3	9	25.0	92	90	91.0
12		4	12	6	6	50.0	92	90	91.0
17		5	17	8	9	47.1	94	88	91.0
17		6	17	10	7	58.8	92	82	87.0
15		7	15	5	10	33.3	96	84	90.0
16		8	16	10	6	62.5	88	76	82.0
16		9	16	11	5	68.7	94	76	85.0
7		10	7	5	2	71.4	106	74	90.0
17		11	17	7	10	41.2	94	72	88.0
Total	......	......	169	72	97	42.6	......	......	......

According to table 2, only 4 of a total of 203 beets developed curly top after being exposed to single previously noninfective beet leafhoppers that had fed on diseased and then on the healthy beet seedlings for periods varying from 2 to 11 hours. The shortest period required for a single leafhopper to transmit curly top was 7 hours. The length of time that each leafhopper transmitting the disease was confined on diseased beets was 6, 8, 5, and 3 hours and on healthy beets 1, 1, 5, and 8 hours, respectively.

TABLE 3

TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS WHICH WERE KEPT FOR VARYING PERIODS ON DISEASED BEETS AND THEN TRANSFERRED HOURLY TO SUCCESSIVE HEALTHY BEETS

Number of single leafhoppers kept on healthy beets	Length of time on curly-top beets, hours	Virus incubation period in leafhoppers in hourly tests	Virus incubation periods in leafhoppers producing infections, hours	Number of beets inoculated	Beets infected	
					Number	Per cent
20	4	16–23	17, 21, 22, 22, 23, 23	160	5	3.1
10	12	13–20	14, 20	80	2	2.5
10	20	20–23	21	40	2	5.0
10	21	22–23	0	20	0	0.0
50	....	13–23	....	300	9	3.0

The transmission of curly top by single infective beet leafhoppers left on healthy beet seedlings for periods of 1 to 3 hours varied from 10.0 to 25.0 per cent, as compared with 33.3 to 71.4 per cent for periods of 4 to 11 hours. The percentage of infection did not constantly vary directly as the time of exposure on healthy beets. Repeated inoculations of the beet seedlings during the longer feeding periods probably increases the amount of virus injected into the beet and explains the higher percentage of infection obtained.

A number of experiments were undertaken varying the exposure of previously noninfective beet leafhoppers on diseased beets and then transferring each insect hourly to successive healthy beets. The hoppers were fasted from 2 to 7 hours in empty cages and were then exposed to diseased beets for periods varying from 4 to 21 hours as indicated in table 3. In the first experiment indicated in table 3, 20 previously noninfective males were fasted for 2 hours, left on a diseased beet for 4 hours, and fasted for 12 hours to avoid transmission of the disease by contamination of mouth parts. It is not known how long the virus remains viable on the mouth parts, but it was assumed that it would not be viable after the insects had been kept in cages without food for 12 hours. In the next three experiments the insects

in cages remained on diseased beets overnight, and the glass of the cage was placed in front of 2 electric lamps provided with reflectors. The transmission of curly top by single insects with a virus incubation period of 13 to 23 hours is shown in table 3.

According to table 3, 50 leafhoppers with an incubation period of 13 to 23 hours transmitted curly top to 9 (3 per cent) of 300 beets. Table 2 shows that 203 beet leafhoppers tested singly, with an incubation period of 2 to 11 hours, transmitted curly top to 4 (2 per cent) of 203 beets. The first set of data were obtained with leafhoppers which fed on diseased beets at night, whereas the second set of data were obtained with insects which were fed on diseased and healthy beets during the daytime and at higher temperatures.

MASS INOCULATION

Carsner and Lackey[3] state that the relation of mass action to curly-top infection has been shown by the following methods: "(1) By varying the amount of inoculated virus by (*a*) inoculations with contrasting numbers of leafhoppers and (*b*) unequal periods of exposure; (2) by use of plants differing in susceptibility; (3) by studies on the incubation of the virus in the insect; and (4) by comparing the minimal infective doses of the virus in its virulent and attenuated conditions." However, no experimental data to support this statement has appeared in print.

Severin[7] published the results of mass inoculation using small numbers of insects. In this work nymphs after feeding singly for 5 minutes on a diseased leaf, were fed 10 minutes on healthy beets with 2 to 7 leaves; 3 nymphs to each leaf, or 6 to 21 to a beet, were used. This experiment was repeated with 12 beet seedlings and 131 nymphs, but not a single case of curly top developed.

A comparison was made of the transmission of curly top by single beet leafhoppers with that by 5, 10, 20, 40, and 80 specimens, using virus incubation periods varying from 12 to 19 hours and from 19 to 24 hours. In the first experiment from 1 to 40 previously noninfective male leafhoppers were fed on a diseased beet for a period of 11 hours and then hourly on 8 successive healthy beets. In the second experiment from 1 to 80 adults were fed on a diseased beet for a period of 4 hours, then fasted for 14 hours, and fed hourly on 6 successive healthy beets. It was assumed that the virus was not viable on the mouth parts after the insects were kept in cages without food for a period of 14 hours. The results are indicated in table 4.

It is evident from table 4 that in these experiments single beet leafhoppers with a virus incubation period varying from 12 to 24 hours failed to transmit curly top when fed 1 hour on each healthy beet; a total of 20 males were fed singly on 140 beets. In all probability, the quantity of virus inoculated into the beets was not sufficient to produce infection. When 5 to 80 leafhoppers were used, the size of the dose of the virus was presumably increased. Infection was produced oftener with 40 to 80 insects than with 5 to 20. It is evident, however, that 40 to 80 insects failed to transmit the disease every hour after the first infection was obtained.

TABLE 4

TRANSMISSION OF CURLY TOP BY 1 TO 80 BEET LEAFHOPPERS WITH VIRUS INCUBATION PERIODS OF FROM 12 TO 19 AND 19 TO 24 HOURS*

Virus incubation period in insects, hours	Number of insects on each beet														
	1	1	1	1	1	1	1	1	1	1	5	10	20	40	80
Leafhoppers fed 11 hours on a diseased beet															
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	....
13	–	–	–	–	–	–	–	–	–	–	–	+	–	–	....
14	–	–	–	–	–	–	–	–	–	–	–	–	–	+	....
15	–	–	–	–	–	–	–	–	–	–	–	–	–	+	....
16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	....
17	–	–	–	–	–	–	–	–	–	–	–	+	+	–	....
18	–	–	–	–	–	–	–	–	–	–	–	–	–	+	....
19	–	–	–	–	–	–	–	–	–	–	–	+	+	+	....
Total positive (+)	0	0	0	0	0	0	0	0	0	0	0	3	2	4	....
Total negative (–)	8	8	8	8	8	8	8	8	8	8	8	5	6	4	....
Leafhoppers fed 4 hours on a diseased beet and fasted 14 hours															
19	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
20	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
21	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+
22	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+
23	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
24	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+
Total positive (+)	0	0	0	0	0	0	0	0	0	0	3	2	3	5	5
Total negative (–)	6	6	6	6	6	6	6	6	6	6	3	4	3	1	1

* The plus sign (+) indicates the production of the disease, and the minus sign (–) shows that no disease resulted.

A comparison was made of the transmission of the curly-top virus by two experiments on lots of 1 and of 5 beet leafhoppers which were kept on diseased beets for the same period, and for varying periods on healthy beets on each of 7 successive days. In the first

experiment previously noninfective males were kept in empty cages for 4 hours, then placed on diseased beets for 7 hours, on Mammoth or Alameda sweet corn, immune to curly top, for 13 hours, and on healthy beet seedlings, for 4 hours during the first day. During the second to the seventh day the insects were kept on sweet corn plants for a period of 20 hours and on healthy beets for 4 hours. In the second experiment the procedure was the same, except that sweet corn plants were not used. The insects were kept on diseased beets for 7 hours and on healthy beets for 17 hours during the first day, and for 24 hours daily on healthy beets during the second to the seventh day. The results are indicated in table 5.

It is evident from table 5 that mass inoculation and the period of exposure of the insects on healthy beets is a factor in the percentage of curly-top transmission. Fifteen previously noninfective males fed singly on 105 beets for a period of 4 hours on 7 successive days transmitted curly top to 8 beets (7.6 per cent), whereas 3 lots of 5 males fed for periods of 4 hours on 21 beets on 7 successive days transmitted the disease to 7 beets (33.3 per cent). Fifteen previously noninfective males fed singly on 105 beets for periods of 17 or 24 hours on 7 successive days transmitted curly top to 32 beets (30.5 per cent) as compared with 16 diseased beets (76.2 per cent) of 21 beets inoculated by 3 groups of 5 males during a 24-hour period.

A comparison was made of the transmission of curly top by 2 lots of 1, 2, 3, 4, and 5 previously noninfective with infective beet leafhoppers during a period of 1 to 7 days. Previously noninfective males were kept for a period of 7 hours on a diseased beet from which the infective leafhoppers used in this experiment had been removed. They were fed for 17 hours on a healthy beet seedling during the first day and 24 hours daily during the second to the seventh day. The infective insects which had completed the nymphal instars on a diseased beet were transferred daily to successive healthy beets for a period of 7 days. The results are shown in table 6.

According to table 6, 15 previously noninfective beet leafhoppers fed singly on 105 beets during a period of 1 to 7 days transmitted curly top to 33 beets (31.4 per cent) whereas 15 infective leafhoppers fed singly on 105 beets during a period of 1 to 7 days transmitted curly top to 65 beets (61.9 per cent). Infective insects, which were reared on curly-top beets, probably contain a larger quantity of the virus than specimens with a virus incubation period of only 1 to 7 days. When 2 previously noninfective and 2 infective insects were used, the average curly-top transmission during a period of 7 days was 66.7 and 81.0 per cent respectively. When 3 previously noninfective and

3 infective leafhoppers were used, both groups of insects infected 76.2 per cent of the beets. When lots of 4 or 5 leafhoppers were used the average infections obtained by the previously noninfective insects was 71.4 per cent compared with 85.7 per cent with the infective bugs.

TABLE 5

COMPARISON OF TRANSMISSION OF CURLY-TOP VIRUS BY TWO LOTS OF 1 AND 5 BEET LEAFHOPPERS DURING A PERIOD OF 1 TO 7 DAYS, BOTH LOTS FED ON DISEASED BEETS FOR 7 HOURS

Number of leafhoppers on each beet	Virus incubation period in leafhoppers, days	Number of beets inoculated	Number of beets infected	Per cent infected
Fed 4 hours daily on a healthy beet during a period of 1 to 7 days				
1	1	15	0	0.0
1	2	15	3	20 0
1	3	15	0	0 0
1	4	15	0	0.0
1	5	15	2	13.3
1	6	15	2	13.3
1	7	15	1	6 7
Total	....	105	8	7.6
Fed 17 hours on a healthy beet during the first day and 24 hours daily during the second to the seventh day				
1	1	15	3	20.0
1	2	15	3	20.0
1	3	15	6	40 0
1	4	15	7	46 7
1	5	15	4	26.7
1	6	15	5	33.3
1	7	15	4	26.7
Total	..	105	32	30 5
Fed 4 hours daily on a healthy beet during a period of 1 to 7 days				
5	1	3	2	66.7
5	2	3	2	66.7
5	3	3	1	33.3
5	4	3	0	0.0
5	5	3	1	33.3
5	6	3	1	33.3
5	7	3	0	0.0
Total	...	21	7	33.3
Fed 17 hours on a healthy beet during the first day and 24 hours daily during the second to the seventh day				
5	1	3	1	33.3
5	2	3	2	66.7
5	3	3	2	66.7
5	4	3	3	100.0
5	5	3	3	100.0
5	6	3	3	100.0
5	7	3	2	66.7
Total	....	21	16	76.2

TABLE 6

COMPARISON OF TRANSMISSION OF CURLY TOP BY TWO LOTS OF 1 TO 5 PREVIOUSLY NONINFECTIVE WITH INFECTIVE BEET LEAFHOPPERS DURING A PERIOD OF 1 TO 7 DAYS

Number of leaf-hoppers on each beet	Virus incubation period in leafhoppers, days	Number of beets inoculated	Number of beets infected	Per cent infected	Number of leaf-hoppers on each beet	Number of beets inoculated	Number of beets infected	Per cent infected
Previously noninfective leafhoppers					Infective leafhoppers			
1	1	15	2	13.3	1	15	7	46.7
1	2	15	5	33.3	1	15	13	86 7
1	3	15	6	40.0	1	15	8	53.3
1	4	15	4	26.7	1	15	9	60.0
1	5	15	6	40.0	1	15	9	60 0
1	6	15	5	33.3	1	15	10	66.7
1	7	15	5	33 3	1	15	9	60.0
Total	....	105	33	31 4	......	105	65	61 9
2	1	3	1	33 3	2	3	1	33.3
2	2	3	2	66.7	2	3	2	66.7
2	3	3	2	66.7	2	3	3	100.0
2	4	3	2	66 7	2	3	3	100 0
2	5	3	2	66.7	2	3	3	100.0
2	6	3	3	100.0	2	3	2	66.7
2	7	3	2	66.7	2	3	3	100 0
Total	....	21	14	66 7	....	21	17	81.0
3	1	3	1	33 3	3	3	1	33.3
3	2	3	2	66.7	3	3	2	66.7
3	3	3	3	100.0	3	3	3	100.0
3	4	3	3	100.0	3	3	3	100 0
3	5	3	2	66.7	3	3	3	100.0
3	6	3	2	66.7	3	3	2	66.7
3	7	3	3	100 0	3	3	2	66.7
Total	....	21	16	76 2	....	21	16	76 2
4	1	3	1	33.3	4	3	3	100.0
4	2	3	1	33.3	4	3	2	66.7
4	3	3	2	66.7	4	3	2	66.7
4	4	3	3	100 0	4	3	3	100.0
4	5	3	2	66.7	4	3	3	100 0
4	6	3	3	100.0	4	3	2	66.7
4	7	3	3	100.0	4	3	3	100.0
Total	....	21	15	71.4	....	21	18	85.7
5	1	3	1	33.3	5	3	2	66.7
5	2	3	3	100.0	5	3	3	100.0
5	3	3	2	66.7	5	3	1	33.3
5	4	3	3	100.0	5	3	3	100.0
5	5	3	1	33.3	5	3	3	100.0
5	6	3	3	100.0	5	3	3	100.0
5	7	3	2	66.7	5	3	3	100.0
Total	....	21	15	71.4	....	21	18	85 7

INCUBATION PERIOD OF VIRUS IN BEET LEAFHOPPER

The virus incubation period in the beet leafhopper should be defined as the time for the infective principle to pass into the mouth parts, alimentary canal, blood, and salivary glands and out of the mouth parts in sufficient quantity to produce infection. The term incubation period should not be used when testing short periods of the transmission of the disease, such as contamination of mouth parts.

Carsner and Stahl[2] demonstrated that a single insect was able to transmit curly top within a period of 21¾ hours. These investigators reported "that a greater number of insects become able to transmit the virus after a longer period than 24 hours than are able to do so in the shorter time. The facts cited seem to indicate that a multiplication of the causal agent takes place within the insects."

The virus incubation period in single previously noninfective insects is given in table 6. The percentage of curly top obtained with single insects varied from 13.3 to 40.0 during virus incubation periods of 1 to 7 days, the lowest percentage occurring at the end of 1 day.

CONTAMINATION OF MOUTH PARTS

An experiment was conducted in which the mouth parts from beet leafhoppers were cut off in a culture medium and fed to noninfective leafhoppers. The mouth parts from 1,000 adults were dissected, 100 being used in each experiment. Previously noninfective adults, after being kept in an empty cage for a period of 6 hours, began to feed on a diseased beet within a few minutes under high temperatures in the greenhouse. After successive lots of 25 insects had fed for periods of ½ to 1 hour, they were captured with a pipette, etherized, and dissected. The labium, maxillae, and mandibles were cut off with a small triangular scalpel on clean slides and put into equal volumes of steam-extracted beet-root juice and sterile distilled water containing 5 per cent beet sugar. The mouth parts in the culture medium were ground in a mortar and fed to noninfective nymphs.

Feeding equipment was devised in which a small quantity of culture medium was employed. Microculture slides were used in which the depression containing the culture medium was 18 mm in diameter and 3 mm deep. Specimen vials (90x24 mm) were cut about an inch below the constriction by nichrome wire heated with elec-

tricity The cut surface below the neck of the vial was flared and covered with fish skin which was held in place by elastic bands below the flare. The nymphs were placed in the cut vial with a pipette, and the mouth was covered with silk bolting. The membrane was now put in contact with the culture medium containing the mouth parts (fig. 4). The feeding equipment was placed on a plate of glass below a bell jar, with a beaker of water to prevent evaporation (fig. 5).

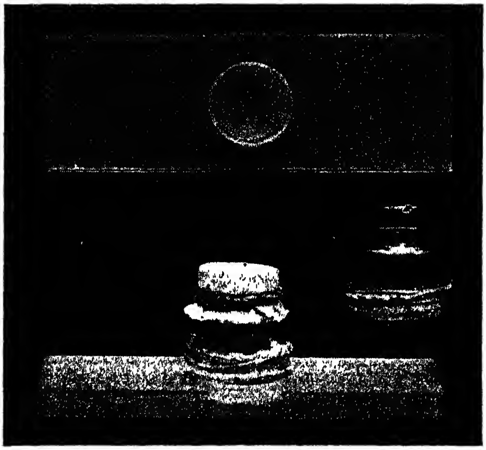

Fig. 4. Equipment used in feeding noninfective nymphs on culture media containing feces or severed mouth parts from infective beet leafhoppers. A 'fish-skin' membrane, covering the flared surface of the glass cage, was in contact with the culture media containing the feces or severed mouth parts in the depression of a microculture slide. The opened end of the cage containing the nymphs was covered with silk bolting.

Noninfective nymphs after feeding on the culture medium containing the mouth parts were transferred to 30 beet seedlings, using about 20 hoppers for each beet. Two typical cases of curly top developed, but the incubation periods of the disease in the beets were 24 and 34 days, respectively. The usual period for symptoms to develop in beet seedlings with 6 to 8 leaves is from 1 to 2 weeks during the summer.

After the mouth parts were dissected in each experiment, the heads, containing the blood and salivary glands, were placed in a culture medium and ground in a mortar. The culture medium containing the crushed heads was centrifuged for about an hour, and either fed directly to noninfective nymphs or after filtering through coarse and fine Berkefeld candles. Noninfective nymphs, after feeding on the filtered and unfiltered culture media, in which the heads without

mouth parts were crushed, failed to transmit curly top to healthy beet seedlings. It appears from this experiment that in feeding noninfective beet leafhoppers on curly top beets for a period of ½ to 1 hour, the virus failed to pass through the wall of the midintestine into the blood and salivary glands.

Fig. 5. The feeding equipment and a beaker of water under a bell jar resting on a glass plate, to prevent evaporation of the culture media containing the feces or severed mouth parts.

FECES

A common method of insect transmission of diseases to plants is through the feces containing spores of fungi or bacteria. "Among fungus diseases, the spores of at least seven parasitic species have been shown capable of passing through the intestinal canal in a viable condition."[6] Infection with bacterial wilt of cucurbits takes place directly from the feces of the striped and twelve-spotted cucumber beetle (*Diabrotica vittata*, and *D. duodecimpunctata*) brought in contact with a leaf injured by the feeding beetles. Among human and animal diseases it has been shown that the feces of infected fleas when applied to the abrasion in the skin produces bubonic plague, and in a similar manner the feces of infected lice produce typhus fever.

Severin[8] failed to transmit curly top by inoculating the feces from infective beet leafhoppers into the petioles of healthy beets.

Carsner and Stahl[2] dipped the point of a steel needle into drops of fresh excreta from the leafhopper and then pricked the excrement into two beet seedlings; no disease resulted.

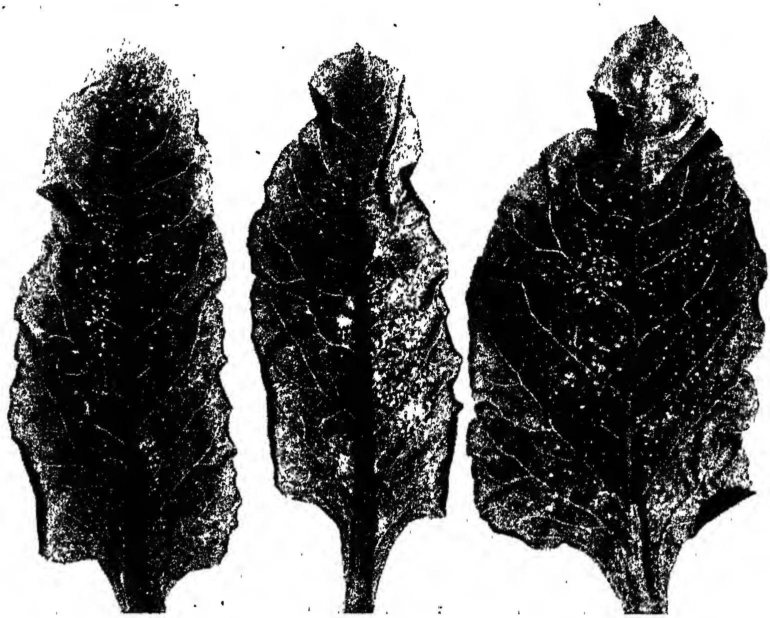

Fig. 6. Mouth-part punctures of beet leafhoppers in blades of sugar beets. The puncture is in the center of the white circular area. Mouth-part punctures are rarely visible to the naked eye, but when large numbers of adults feed on the thin younger leaves, an occasional leaf may show the white circular areas.

When large numbers of beet leafhoppers are reared on beets, mouth-part punctures are common in the foliage, as may be seen by examining it with a binocular miscroscope. Mouth-part punctures are rarely visible to the naked eye but when large numbers of adults feed on the thin younger leaves, an occasional leaf may show white circular areas with the puncture in the center (fig. 6). If some of the curly-top virus passes through the digestive canal of the hopper in a viable condition, and the excreta is spurted into the feeding punctures, the virus might possibly thus enter the phloem of the beet leaves.

Since juice pressed from the leaves and roots of curly-top beets, when inoculated into the crown of healthy beets, produced such a low percentage of infection,[10] it was decided to feed noninfective beet

leafhoppers on the feces of infective hoppers, by the methods described by Carter[4, 5] and Severin and Swezy.[12]

During the past two years about 12,000 infective beet leafhoppers were confined at the rate of about 500 adults to each sterile test tube for periods varying from 10 to 60 minutes; the feces were then washed from the test tubes with various culture media. In some experiments the culture medium containing the excrement was fed directly to noninfective nymphs, while in others the hoppers fed on the filtrate prepared from the feces while fresh or after it had been incubated for 1 to 6 weeks. The nymphs were then transferred to 91 beet seedlings but no curly top developed. Further experiments are necessary to determine whether the feces from infective beet leafhoppers are toxic to the curly-top virus.

DISCUSSION

Regurgitation of food is common among insects. Insects sometimes vomit after overfeeding or after taking in poisoned food. It has been demonstrated that disease-producing microorganisms which have been taken up by insects were in an infective state in the vomit spots.

Swezy[14] found only 1 beet leafhopper in 250 specimens examined that had two lumps of bacteria in the esophagus anterior to the esophageal valve (fig. 1). It was assumed that this abnormal condition of the alimentary canal would explain the occurrence of infection in ½ to 1 hour. There is no evidence to show, however, that a single previously noninfective beet leafhopper after feeding on a diseased beet can transmit curly top in these short periods.

If an obstruction in front of the esophageal valve occurs in the beet leafhopper, or if the hopper gorges the midintestine with food in overfeeding under high temperatures, so that the esophagus becomes greatly distended, then after the withdrawal of the mouth parts, some of the beet juice should flow from the foreintestine through the canal in the setae and accumulate as a droplet at the end of the labium. A droplet of beet juice at the end of the beak has never been observed with the work on short periods of curly-top transmission and other feeding experiments. Droplets of excrement are plainly visible to the naked eye, but in hundreds of experiments extending over a period of thirteen years, a leafhopper with a drop of beet juice at the end of the labium has never been observed.

If a beet leafhopper is vivisected and the contractions of the alimentary canal are studied, the movement of the esophageal valve is

always a swallowing movement as has been described in other insects. A peristaltic movement of the foreintestine aided by a swallowing movement of the esophageal valve, should force a plastic mass of bacteria through the valve into the midintestine. The esophageal valve, however, prevents regurgitation from the midintestine into the fore-intestine.

Two other theories were proposed by Swezy[14] to account for the short periods of curly-top transmission by the beet leafhopper (1) by the passage of the infective organism unchanged through the body of the insect, and (2) by a change and completion of a life cycle of an infective organism.

It has not been proved, however, that the curly-top virus can pass through the body of the leafhopper quickly enough to account for curly-top transmission in short periods. In the experiments reported in this paper, the curly-top virus was not obtained from filtrates prepared from crushed heads of the leafhoppers with the mouth parts removed, when the insects had fed only ½ to 1 hour on curly-top beets. Curly-top transmission was obtained, however, with the cultures prepared from mouth parts of large numbers of insects which had fed on curly-top beets for only ½ to 1 hour.

These experiments indicate that contamination of mouth parts, without multiplication of the curly-top virus in the body of the insect may account for the transmission of the disease in short intervals.

Curly-top transmission in short intervals by single insects feeding on diseased beets and then on healthy beets has not been accomplished up to the present time. The quantity of virus washed by the saliva from the mouth parts of large numbers of leafhoppers into the feeding punctures is probably a factor in the transmission of the disease. When juice is extracted from the blades and petioles of curly-top beets, a rapid inactivation of the virus occurs, but whether this is the case with the virus on the mouth parts when withdrawn from the phloem is not known. The season of the year and temperature are factors in the short periods of curly-top transmission by the beet leafhopper.

SUMMARY

If an experiment on short periods of transmission, 40 previously noninfective beet leafhoppers after feeding on a diseased beet transmitted curly top to a healthy beet within 20 minutes. The percentage of curly-top transmission varied with the time that the healthy beet was exposed to infection as follows: 20 minutes, 2.4 per cent; ½ hour,

14.3 per cent; 1 hour, 2.9 per cent; 1½ hours, 16.7 per cent; 2 hours, 15 per cent; 3 hours, 27.8 per cent; and 4 hours, 33.3 per cent. When 20 to 50 adults were used, the transmission of the disease within 20 minutes to 4 hours averaged 7.3 per cent with 96 beets, as compared with 22.5 per cent with 129 beets when more than 50 hoppers were used. Curly top was not transmitted when 5 to 15 insects were used in short feeding intervals.

A number of experiments were performed varying the time of exposure of single previously noninfective beet leafhoppers on diseased and healthy beets with the following results: a total of 203 leafhoppers, after feeding singly on diseased and healthy beet seedlings for periods varying from 2 to 11 hours, transmitted curly top to only 4 (2.0 per cent) of 203 beets. The shortest period for a single insect to transmit curly top was 7 hours. Fifty leafhoppers with a virus incubation period of from 13 to 23 hours, tested singly, transmitted curly top to 9 (3 per cent) of 300 beets. In another experiment 20 males with a virus incubation period of from 12 to 24 hours, fed singly on 140 beets, failed to transmit curly top.

When lots of 5, 10, 20, 40, or 80 leafhoppers fed hourly on different healthy beets, the size of the dose of the virus was increased; infection was produced oftener with 40 or 80 insects than with 5, 10, or 20 hoppers.

The relation of mass inoculation by groups of beet leafhoppers to curly-top transmission was demonstrated by varying the time of exposure of the insects on healthy beets. Fifteen previously noninfective males fed singly on 105 beets for a period of 4 hours on 7 successive days transmitted curly top to 8 beets (7.6 per cent) whereas 3 lots of 5 males fed for periods of 4 hours on 21 beets on 7 successive days transmitted the disease to 7 beets (33.3 per cent). Fifteen previously noninfective males fed singly on 105 beets for periods of 17 or 24 hours on 7 successive days transmitted curly top to 32 beets (30.5 per cent) compared with 16 diseased beets (76.2 per cent) by 3 groups of 5 males during a 24-hour period.

The percentage of curly top transmitted by single insects with virus incubation periods of 1 to 7 days varied from 13.3 to 40 per cent, the lowest percentage occuring at the end of 1 day. The exposure of each male on a diseased beet was 7 hours, and on each healthy beet 17 hours during the first day and 24 hours during each of the next 6 days.

The mouth parts were contaminated with the curly-top virus after the leafhoppers had fed on a diseased beet for periods of ½ to 1 hour.

The mouth parts were cut off, put in a culture medium, and ground in a mortar. Previously noninfective nymphs after feeding on the culture medium transmitted curly top to 2 beet seedlings but the incubation period of the disease in the beet was prolonged, requiring 24 and 34 days, respectively.

Noninfective nymphs after feeding on culture media containing the excreta or on the filtrate prepared from the feces failed to transmit curly top to healthy beets.

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THE INFECTIOUS NATURE OF POTATO CALICO

D. R. PORTER[1]

The nature of potato calico, a degeneration disease of the Irish potato, has been under investigation at the California Agricultural Experiment Station during three seasons. The disease has been observed in every important potato-producing district in the state, from San Diego County, in the extreme southern part, to Humboldt County, in the extreme north. It was prevalent in certain fields in the Delta region in 1929–1930; and growers in San Bernardino, Riverside, Tulare, and Kern counties have stated that calico is steadily increasing in prevalence in their fields, even though they continue to plant only their own seed stock. The evidence presented herewith establishes the infectious nature of potato calico, adding one more virus disease to the present list. All experiments and observations reported herein were carried out with the potato variety White Rose, or, where indicated, with seedlings.

HISTORICAL

In 1920, Hungerford[(7)] described potato calico as a non-infectious disease and in later reports[(8) (18) (19)] brought out that the disease was tuber-perpetuated; that the yield reduction by calico was slight, if measurable at all; and that chlorophyll deficiency was less pronounced at blossoming time than in the early life of the diseased plant. He further observed that about three per cent of the plants were infected and that the disease was commonly found in irrigated fields. Although he did not consider the disease a serious one, he recommended that diseased plants should be removed from the field.

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In Washington, Dana[3] [4] [5] observed apparent spread in the field, redemonstrated tuber perpetuation, and reported negative results with transmission by means of tuber grafts, or aphids. In 1926[12] it was reported from Oregon that calico was among the virus diseases which had been identified in that state. Young and Morris[20] reported calico from Montana in 1929, and in 1931[21] reported unsuccessful attempts to transmit the disease by tuber grafting; they found that the disease was tuber-perpetuated and that infected plants produced low yields. They suggested that infected plants should be rogued. McKay and Dykstra[10] temporarily classified calico as a virus disease, although they obtained only one positive case of transmission. They reported that diseased plants were stunted, that tuber perpetuation did not always occur, and that the increase in regional prevalence suggested the infectious nature of the disease.

NATURAL FIELD SYMPTOMS

Some of the leaflets of infected plants growing in the field become irregularly spotted or blotched. These areas do not become necrotic; rather they appear devoid of chlorophyll and generally assume a bright brilliant-yellow, yellowish-white, or gray color. The spots are not always inter-veinal and may occupy as much as 95 per cent of the leaflet area, being, as a rule, irregularly scattered. As a probable result of chlorophyll deficiency, plants infected when young seldom attain normal size. If infected when nearly mature, they show no significant stunting. If more than 50 per cent of the plants in the field are infected, the crop appears from a distance to be diseased. Under conditions of close planting, such as is commonly practiced in the Delta region, non-infected plants often grow over and obscure plants which were infected when small. The symptoms of calico as they appear on infected leaflets in the field are shown in plate 1 (lower).

INOCULATIVE SYMPTOMS

The young leaflets of healthy[2] plants which have been artificially inoculated with unfiltered juice extracted from infected plants assume symptoms practically identical with those observed in the field; but, in

[2] In this paper any reference to healthy plants indicates plants produced by tubers which had been indexed and found free of calico. Such stock was likewise free from visible symptoms of other virus diseases. Indexing was accomplished by planting the stem-end bud of each tuber in steamed peat in the greenhouse. If the resulting plant was visibly free of calico or other known virus diseases, it was assumed that the remainder of the indexed tuber was likewise virus-free.

addition to yellowing, the lower leaflets may become necrotic at the tip, crinkled or ruffled, and in some instances slightly stiffened. Plants inoculated when 15 inches tall rarely exhibit yellowing of the lower leaflets or of those which were inoculated. Artificially infected plants are stunted; the leaflets are smaller and the leaves shorter than normal. Under certain undefined environmental conditions, diseased leaflets may regain their normal color as they age. The stems are usually smaller on diseased than on non-infected plants produced by the same tuber. Inoculative symptoms produced on a seedling plant are shown in plate 1 (upper).

SPREAD IN THE FIELD

Much circumstantial evidence suggests the infectious nature of potato calico. In September, 1929, tubers were harvested from infected plants and from adjoining plants apparently calico-free. These tubers were cut with a flamed knife, and planted in the field at Davis on March 25, 1930. The progeny of the infected plants harvested in 1929 showed a higher per cent of calico in the field in 1930 than the progeny of the apparently calico-free plants which adjoined the infected plants in 1929. The progeny of the apparently calico-free plants, however, manifested enough calico in the field in 1930 to suggest that transmission had occurred in the field in 1929. Data from this trial appear in table 1. Tubers from the 1930 crop were planted the following winter in the greenhouse. The results of the greenhouse planting are also given in table 1.

TABLE 1

Relative Per Cent Calico Produced by Tubers from Infected and Healthy-Appearing Plants Grown Near Infected Plants in the Field

Seed stock, grown in 1929	Number of progeny plants in the field, 1930	Per cent infected plants in the field 1930			Per cent infected plants in second generation in the greenhouse 1930-31
		May 6	May 28	July 6	
A, tubers from calico-infected plants	12	83	83	83	85
B, tubers from non-infected plants adjacent to A in the same row	40	15	20	25	56
C, tubers from non-infected plants in the rows adjoining those of infected plants, A	48	0	11	11	49

Further circumstantial evidence of calico transmission was obtained in the field at Davis in 1930. Calico-infected stock was planted in one row (row 57) across the field; and four rows on either side (rows 53 to 56, inclusive, and 58 to 61, inclusive) were planted with healthy stock. The healthy stock planted in rows 58 to 61 was also planted in isolated fields at San Jose, Shafter, and Temecula; and, as no calico developed in these distant plots, the evidence indicates that this stock was free of calico when planted at Davis. The fact that calico appeared in this healthy stock in the field at Davis indicates current-season transmission of the disease in the field in 1930. Data from this trial are presented in table 2. It appears that a higher per cent of calico was present in the rows of healthy stock nearest row 57, and that slightly less transmission occurred in rows 53 and 61, each situated 14 feet from row 57.

TABLE 2

INDICATIONS OF CURRENT-SEASON TRANSMISSION OF POTATO CALICO IN THE FIELD AT DAVIS, 1930, WHERE FOUR ROWS OF HEALTHY STOCK WERE PLANTED ON EITHER SIDE OF A ROW PLANTED WITH CALICO-INFECTED STOCK

Row No.	Stock planted	Distance in feet from calico-infected stock (row 57)	Per cent infected plants		
			May 6	May 28	June 11
53	Healthy	14.0	0.0	1.25	3 75
54	Healthy	10.5	0.0	6.25	6 25
55	Healthy	7.0	0.0	7.50	7.50
56	Healthy	3.5	2.5	12.50	13.75
57	Calico-infected	....	27.1	38.50	40.30
58	Healthy	3.5	?	15.00	15.00
59	Healthy	7.0	?	12.50	13.75
60	Healthy	10.5	?	5.00	6.25
61	Healthy	14.0	?	7.50	10.00

White Rose stock, here designated as lot D, grown in Minnesota in 1929 in a place where no calico was seen,[3] was planted in 1930 in the greenhouse at Davis, and by a grower in the Delta region near Stockton. In the greenhouse no calico was evident on any of 240 plants, each produced by a different tuber—a fact indicating that lot D was free of calico.

The plantings of lot D in the Delta were made in two places. Field No. 1 was situated near the levee, the north slope of which was cropped with alfalfa. In this field lot D was planted in two 40-foot

[3] J. J. Thompson, seed expert with Zuckerman Brothers, who inspected lot D growing in Minnesota, saw no calico there either in 1929 or in 1930.

strips, each running parallel with the levee and separated from one another by a seven-foot space, as shown in figure 1. Four feet of this space was occupied by an irrigation ditch. The plot between the levee and the first ditch was designated as D and that beyond the first

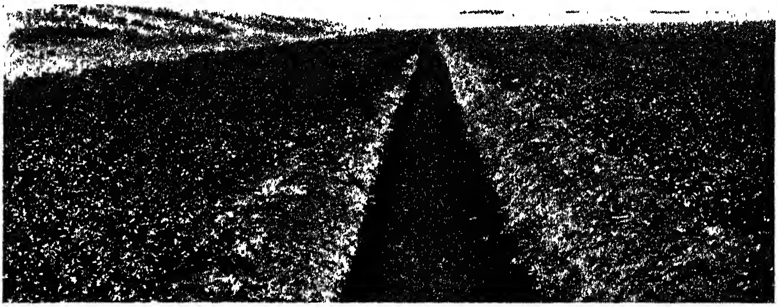

Fig. 1. The irrigating ditch in the center separates seed lot D on the left from lot D*a* on the right. Note alfalfa plants on the high levee on the extreme left. A condition suggestive of calico was observed on some of these and on certain weeds in this field in 1930.

Fig. 2. The ditch separates seed lot D*a* on the left from lot E on the right.

ditch as D*a*. Another irrigation ditch separated lot D*a* from a third lot, E, as shown in figure 2. The seed stock for lot E also came from an apparently calico-free field in Minnesota, though not from the same source as D. Calico was first observed in lot D; and, as shown in table 3, the disease seemed to spread from lot D across the first ditch

into lot D*a* and thence into lot E. Although there was 16 per cent calico in lot E, close to D*a*, on July 2, it was observed that calico infection in lot E decreased with increasing distance from lot D*a*, there being only a trace at a distance of 200 feet from D*a*. The data in table 3 indicate the increase in prevalence of calico in these three lots during the summer and suggest natural spread in the field.

TABLE 3

INDICATION OF CURRENT-SEASON SPREAD OF POTATO CALICO IN TWO FIELDS AT STOCKTON, 1930

Stock	History of stock	Location in field, 1930	Per cent infected plants			
			May 13	May 23	June 13	July 2
D	Grown at Thief River Falls, Minn., in 1929. No calico seen.	Adjacent to levee and alfalfa	18	63	91	...
D*a*	Same as D.	Separated by a seven-foot space from D.	trace	11	46	....
E	Grown in Minnesota in 1929. No calico seen	Separated by a seven-foot space from D*a*	none	trace	13	16
D*b*	Same as D and D*a*	About one mile from D	?	trace	trace	1

Lot D*b* (identical in seed stock with lots D and D*a*) was planted in field No. 2 on the same ranch but about one mile from the levee and from field No. 1. Only a trace of calico was observed in the field on June 13, when 91 per cent infection was present in lot D. The results in this field are included also in table 3. Thus, lot D when grown at Davis or at some distance from the levee at Stockton, was comparatively free from calico—a fact indicating that probably no infection had been carried in the seed grown in Minnesota. The apparent spread to lot D near the levee indicated that primary infection probably had come from some source other than the seed. An abnormality suggestive of calico was observed on volunteer potato plants and on certain weeds in the field, as well as on alfalfa growing on the levee. Juice from such diseased alfalfa plants was used in inoculating potato plants, but no calico developed. Insect vectors may have served as agents of transmission, for insects of the genera *Cicadula*, *Eutettix*, *Empoasca*, and *Agallia*, as well as plant lice, were found on calico-infected potato plants. Alfalfa plants have been inoculated with the infectious principle, but calico-like symptoms have not developed. There is no experimental evidence to indicate that alfalfa is susceptible to calico infection, even though the symptoms on alfalfa resemble those on potato.

On June 4, 1930, tubers were dug from 50 plants of lot D, which manifested moderate infection (table 4) and from 50 plants which appeared free of calico infection. On November 19, a number of tubers of each of these two lots were indexed, and the sets were planted in steamed peat in five-inch pots in the greenhouse. The per cent of calico-infected tubers thus tested appears in table 4 and indicates that the progeny of the "apparently calico-free" plants were not so thoroughly infected as the progeny of the "moderately infected" plants. The "apparently calico-free" plants may have been infected; but at the time of digging the tubers were immature, the plants were still green and vigorous, and the infectious principle may not have been diffused into the tubers by that time.

TABLE 4

Per Cent Calico-Infected Plants Produced by Tubers from Moderately Infected and Apparently Calico-Free Plants of Lot D, Grown in the Greenhouse

Stock	Number of tubers indexed	Per cent infected plants in the greenhouse		
		January 5	January 15	January 24
Moderately infected with calico in the field....	48	27.0	43.2	58.5
Apparently calico-free in the field....................	24	18 2	21.4	31.2

TABLE 5

Apparent Transmission of Potato Calico at Stockton, 1930, as Indicated by Tuber Indexing in the Greenhouse

Description of stock indexed	Number of tubers indexed	Per cent infected plants in the greenhouse				
		January 5	January 9	January 15	January 24	February 9
One tuber from each plant infected with calico in the field..................................	16	27	36	46	60	86
One tuber from each plant adjacent to the infected plants, grown in the same row..	34	15	22	26	50	64
One tuber from each plant nearest to the infected plant but in adjoining rows..	32	0	4	16	41	56

For further tests of field spread, tubers were harvested from 17 calico-infected plants growing in the Delta region. The plants came from seed stock grown in Minnesota in 1929. Tubers were also harvested from the four nearest apparently calico-free plants. The progeny of these 85 plants were planted in the greenhouse at Davis in January, 1931. Calico infection is recorded in table 5, and shows

that the per cent of infection was higher in the progeny of the 17 infected plants than in the others. There is also slight evidence that spread was more complete to plants in the same row than to those in adjoining rows. The normal distance between plants in the row was 10 inches, while that between rows was 32. Possible mechanical transmission might be more complete from plant to plant in the row than between rows. Such relative spread is evident from the data in both tables 1 and 5.

ARTIFICIAL TRANSMISSION

Tuber Grafting.—Many attempts to transmit calico through the medium of tuber grafts have been made, but a very low per cent of infection has been obtained by this method. In 1930, Dr. E. S. Schultz tuber grafted 25 Green Mountain half-tubers with tissue from tubers produced by calico-infected plants (secured in California), and planted them at Presque Isle, Maine. In a letter dated September 25, 1930, Dr. Schultz stated that 2 of the 25 tuber grafts produced plants which manifested symptoms of calico, while the ungrafted sister half-tubers produced calico-free plants. Although the results of this experiment suggested the infectious nature of the disease, Dr. Schultz considered the per cent of infection too low for adequate proof. Tuber grafting experiments conducted by the writer have, in general, resulted in failure to transmit the disease. Failure to obtain a high per cent of infection might result from incomplete diffusion of the infectious principle into tubers produced by infected plants, for, as indicated by McKay and Dykstra,[10] and in this paper, neither all the tubers from an infected plant nor all the buds of an infected tuber produce visibly infected plants. Thus, unless the infectious principle is definitely known to be completely diffused in a tuber used for grafting into calico-free tubers, such incomplete diffusion might account for the nature of the results obtained by Dr. Schultz, and those reported herein.

In another trial, two indexed tubers produced plants definitely infected with calico, in the greenhouse in January, 1931, and tissue from the mother seed-pieces was used for core grafting 12 healthy White Rose tubers. The grafted tubers were planted in steamed soil in five-inch pots in the greenhouse. Controls consisted of sister sets of ungrafted healthy tubers. None of the plants produced by grafted tubers developed calico—a fact indicating that either tuber grafting often fails to transmit the disease or that the infectious principle

possibly does not persist in an old seed-piece after it has produced a diseased plant.

Two other tubers which, when indexed produced calico-infected plants, were used for grafting into five healthy White Rose tubers on January 11, 1931. The grafted half-tubers and the sister-pieces used as controls were planted in steamed peat in five-inch pots. Only one of the plants produced by the grafted tubers became infected, suggesting that calico may be transmitted by core grafting. The per cent of transmission in this trial was approximately equal to that obtained in the experiment of Dr. Schultz. The results secured in this trial are not considered as adequate proof of transmission by tuber grafting.

TABLE 6

TRANSMISSION OF POTATO CALICO BY LEAFLET MUTILATION OF SEEDLING POTATO PLANTS; INOCULATED JANUARY 9, 1931, IN THE GREENHOUSE

Method of inoculation	Number of plants inoculated	Per cent infected plants					Ave. plant height, inches		
		Jan. 30	Feb. 2	Feb. 6	Feb. 15	Feb. 21	Jan. 9	Feb. 6	Feb. 21
Sterile distilled water applied with cheesecloth to leaflets (see text)	10	0	0	0	0	0	6.3	13.4	17.6
Needle pricks through infectious juice into leaflets	19	11	21	37	37	37	6.6	12.2	13.1
Leaflets rubbed with fingers moistened with infectious juice	8	12	37	75	75	87	6.8	11.3	12.6
Leaflets rubbed with cheesecloth saturated with infectious juice	4	25	50	50	75	75	6.1	12.1	13.0

Leaflet Inoculation.—While the disease has not been transmitted by means of filtered juice, successful inoculations have been made with *unfiltered* juice from infected leaves. On January 9, 1931, plants produced by true potato seed were inoculated with juice from calico-infected leaves by the use of three methods, as follows: (1) a drop of infectious juice was placed on the upper surface of the leaflet, and 50 small needle pricks were made through this juice into the tissue; (2) the fingers were moistened with infectious juice, and the leaflet was rubbed so as to cause surface injury; (3) sterilized cheesecloth was saturated with infectious juice, and the upper surface of the younger leaflets was rubbed so as to injure the epidermal cells and leaf hairs. Controls were inoculated as above with sterile distilled water. The results of this trial, appearing in table 6, indicate that the

disease is of an infectious nature and can spread through the medium of *unfiltered* juice from infected leaves. On February 11, juice was extracted from the infected plants, indicated in table 6, and three potato seedlings were inoculated. These manifested calico symptoms on February 28. Thus the disease has been transmitted from naturally-infected plants to seedlings and then transferred from these to other seedlings. Comparison of controls and inoculated foliage is shown in figure 3 and on seedlings in figures 4 and 5.

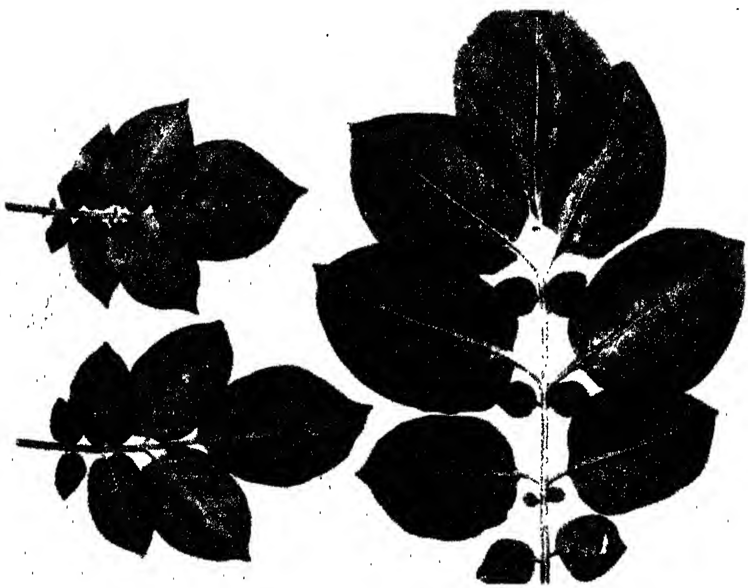

Fig. 3. Calico symptoms on White Rose leaflets (on the left) resulting from mechanical transmission by the use of cheesecloth saturated with *unfiltered* infectious juice. Healthy leaflets (on the right) from a plant inoculated in like manner with sterile distilled water.

Similar results were obtained when healthy plants (from tubers) were inoculated. In this trial healthy White Rose plants were inoculated by leaflet mutilation when approximately eight inches in height, small needle pricks or saturated cheesecloth being used. One hundred per cent infection was obtained 34 days after inoculation by the use of cheesecloth, while needle pricks induced 60 per cent infection after 36 days. In addition to the relatively high per cent of infection, those plants which manifested calico symptoms were stunted when compared with the controls on February 7, as shown in table 7.

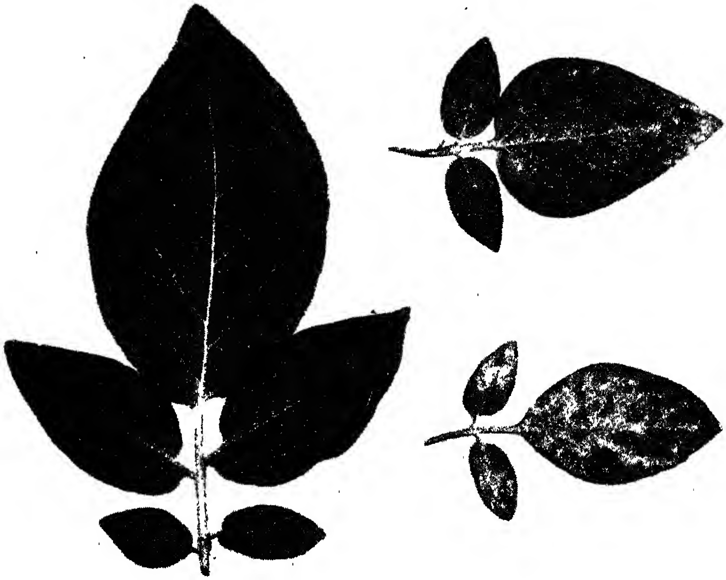

Fig. 4. Right, potato calico on foliage of seedling plants artificially inoculated with *unfiltered* juice. Left, from a seedling plant inoculated with sterile distilled water.

Fig. 5. Various degrees of calico infection on leaflets of potato seedlings artificially inoculated with *unfiltered* juice. Reading from left to right: mild, moderate, and severe types.

TABLE 7

TRANSMISSION OF POTATO CALICO BY LEAFLET MUTILATION OF HEALTHY WHITE ROSE PLANTS; INOCULATED JANUARY 10, 1931, IN THE GREENHOUSE

Method of inoculation	Number of plants inoculated	Per cent infected plants					Average plant height, inches			
		Jan. 30	Feb. 13	Feb. 15	Feb. 17	Feb. 24	Jan. 10	Jan. 30	Feb. 7	Feb. 24
Sterile distilled water applied with sterile cheesecloth..................	5	0	0	0	0	0	8.0	15.1	30.4	32.6
Needle pricks through infectious juice.............	5	0	40	60	60	60	8.5	14.5	25.0	26.1
Cheesecloth saturated with infectious juice....	5	80	100	100	100	100	8.1	14.2	23.0	23.8

EFFECT OF CALICO ON YIELD

Controlled experiments to determine the effect of calico on yield have not been conducted; but it has been observed in the field that infected plants usually produce lower yields than adjacent, non-infected plants. In September, 1929, infected plants were selected at random in a field of about 200 acres, and the tubers of each plant were weighed, and the relative yields compared. It was found that there was a consistent tendency for infected plants to yield less than non-infected plants. Expressed numerically, this reduction in yield was 31 per cent. In September, 1930, this experiment was repeated; the yield decrease was 16 per cent.

In January, 1931, several healthy tubers were quartered and planted in a greenhouse bed in peat that had been steamed for three hours at 30 pounds pressure. When two weeks old, two plants produced by each tuber were inoculated with calico, two sister plants being left as controls. The inoculated plants became infected while the controls remained calico-free. When the tubers were dug in late March, the total yield produced by infected plants was found to be 19 per cent less than that produced by non-infected plants. This figure is probably more reliable than that determined in the field, for sister seed-pieces were used, and contamination with other virus diseases was reduced to a minimum by tuber indexing, through insect control by frequent fumigation of the greenhouse, and by the utilization of steamed soil for the experiment.

COMPARISON OF CALICO WITH SOME SIMILAR POTATO DISEASES

In addition to potato calico, similar diseases manifested by some type of foliage yellowing have been described. Some of these, e.g., aucuba mosaic, yellow top, yellow dwarf, and psyllid yellows, are herewith described, and some of their differential characteristics are listed in table 8. Aucuba mosaic, yellow top, and calico have been artificially transmitted.

Calico.—Hungerford[8] described calico as follows: "This disease is characterized by a pronounced variegation of the leaves of the plant. In extreme cases as much as half of the surface of the leaf may be almost entirely lacking in chlorophyll. The plants appear normal in every other way. Calico is much more pronounced early in the season, many of the leaves appearing to develop chlorophyll in these chlorotic areas at about blossoming time. All evidence to date seems to show that this condition is heritable but not infectious. . . . . . When tubers from plants showing this variegation were planted in the greenhouse, the symptoms which developed were similar to those noted in the field except that in some cases the chlorotic areas turned brown. . . . ."

Aucuba Mosaic.—Quanjer[13] in 1922, described an infectious potato disease which he named aucuba mosaic because the leaf symptoms resembled the variegation of the foliage of *Aucuba japonica*. Later work by Quanjer[14] and Atanasoff[1] established the following facts: (a) the symptoms on the leaflets are manifested by conspicuous yellow spots, usually more or less round and regular in outline; in extreme cases, when these spots coalesce, half of the surface of the leaflet is lacking in chlorophyll; (b) symptoms may be induced in the leaves of plants produced by tubers into which has been grafted other tuber tissue which manifested a certain type of necrosis; (c) transmission may be induced after leaflet mutilation. While the primary symptoms of aucuba mosaic are manifested by small, round spots, the primary symptoms of calico are manifested by irregular, often large areas of yellow, chlorophyll-deficient tissue. Net necrosis of the tuber is reported as a symptom of aucuba mosaic but does not appear to be associated with calico. Comparison of inoculative symptoms of aucuba mosaic and calico is shown in figure 6.

TABLE 8

SOME DIFFERENTIAL CHARACTERISTICS OF CERTAIN POTATO DISEASES* MANIFESTED BY FOLIAGE YELLOWING†

Characteristics	Aucuba mosaic	Psyllid yellows	Yellow top	Yellow dwarf	Calico
Infectiousness	Positive	Probably negative	Positive	Probably positive	Positive
Tuber perpetuation	Positive	Negative	Positive	Positive	Positive
Transmission by: Insects	?	Induced by insect	?	?	?
Transmission by: Leaflet mutilation	Positive	?	?	?	Positive
Transmission by: Tuber grafts	Positive	?	Positive	?	Probable
Dwarfing	None	Extreme	Distinct	Distinct	Moderate
Spindliness	Absent	Mild	Distinct in sprouts	More stocky than normal	Mild
Rolling of leaflets	Absent	Distinct	Distinct at times	Distinct	Absent
Type of leaflet yellowing	Small, lemon-yellow round spots which may coalesce to form irregular blotches	Basal portion of young leaflet has yellowish tinge. Older leaves become yellow and die	Extreme chlorosis at times	Yellowish tinge	Large irregular yellow to cream-colored or gray spots or splotches
Tuber symptoms	Net necrosis at times	None	Net necrosis. Often many small tubers	Often sessile, small, few, and cracked. Flesh discolored as rusty specks. Prominent lenticels	None
Effect on yield	Slight, if any	Reduced	?	Reduced	Reduced
Premature death	No effect	Longer lived	Distinct	?	Very slight

* Exclusive of those known to be caused by microorganisms.

† This comparison would be more accurate if all five diseases could be studied on one clon and under identical environmental conditions. Such has not been possible, and the table was prepared from data and descriptions in the literature, except for calico.

Yellow Top.—According to Folsom,[6] yellow top is manifested by distinct dwarfing, by spindliness, sometimes by extreme chlorosis, sometimes by distinct rolling, by stiff leaf texture, and sometimes by tuber net necrosis, and is tuber-perpetuated. He found that the disease was transmissible by means of grafts.

Fig. 6. Inoculative symptoms of aucuba mosaic on seedling, left, and of calico on White Rose, right. White Rose manifests calico symptoms identical with those of calico on seedlings.

Yellow Dwarf.—Barrus and Chupp[2] described in 1922 a non-infectious disease which was named yellow dwarf by Dr. F. M. Blodgett. Infected plants manifested a dwarfed condition and yellow color, and tubers produced by infected plants were usually small, deformed, deeply cracked, and were often sessile.

Psyllid Yellows.—Recently Richards[15] [16] has reported a serious non-infectious potato disease in the northwest. Because of its association with nymphs of the psyllid, *Paratrioza cockerelli,* and because of the yellowing of the foliage of infected plants, the disease is known as psyllid yellows. Infected plants are severely stunted. There is an upward rolling of the basal portion of young leaves, this rolled portion becoming light pink, yellow, or purple in color. Axillary buds are stimulated into one or a combination of three types of growth: thick

shoots which may exceed the leaf in length, aerial tubers, and rosettes of small and frequently highly colored leaves. In Utah[16] tubers from diseased plants produced normal plants, but in California[17] results which indicated tuber-perpetuation were secured.

SUMMARY

Potato calico is an infectious disease, manifested by irregular blotches of various shades of yellow on the leaflets of infected plants. Inoculative and perpetuation symptoms appear identical. The disease is tuber-perpetuated. Plants artificially infected are stunted.

Natural spread in the field, indicated by inoculative symptoms on healthy plants, has been observed.

The distance, direction, and rapidity of spread in the field and the natural increase in prevalence in certain regions suggest that insects may serve as vectors.

There is questionable evidence that the disease may be transmitted by tuber grafting.

Infection results when healthy leaflets are inoculated with *unfiltered* juice taken from calico-infected plants. The minimum incubation period is about 15 days.

Calico symptoms have not developed in the foliage of healthy plants inoculated with filtered juice of calico-infected plants.

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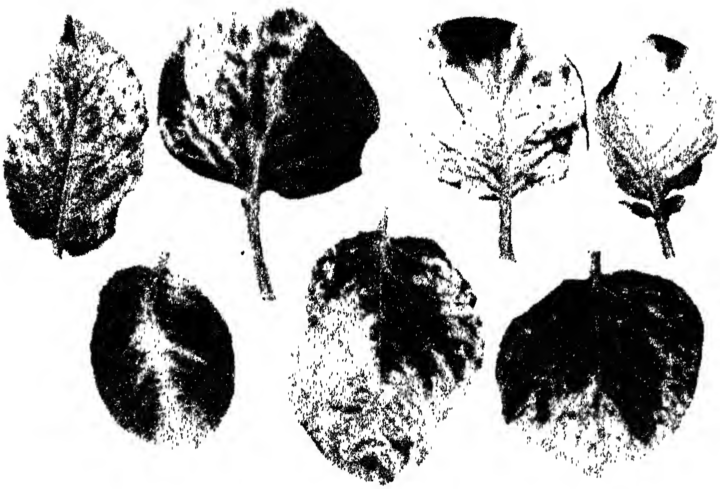

Upper: Potato seedling to which calico was mechanically transmitted by use of sterile cheesecloth saturated with *unfiltered* infectious juice.

Lower: Natural calico-infection of leaflets of White Rose potato. Note the irregular yellowed areas, their uneven distribution, and the faded areas in some of the leaflets.

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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY OF SEVERAL PRESERVATIVES TO MICROORGANISMS

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In the preservation of food products commercially by means of sodium benzoate or of sulfurous acid, marked irregularities in preservative effect have been observed. In experiments conducted in this laboratory several years ago at the request of the industries concerned, it was found that 1/10 of 1 per cent sodium benzoate failed to prevent the spoiling of ripe olives, artichokes, avocado pulp, and sliced avocadoes in brine, whereas more acid products, such as fruit juices and green olives, were preserved satisfactorily by this concentration of benzoate. In other experiments it was found that potassium metabisulfite ($K_2S_2O_5$, the anhydride of $KHSO_3$) was a much less effective preservative for juice from overripe grapes than for juice from slightly immature grapes. It appeared, therefore, that sodium benzoate and potassium metabisulfite are more effective as preservatives in media of high acidity than in those of low acidity.

Because of these and other observations, it was suspected that the reaction of the medium, that is, its hydrogen-ion concentration, probably plays an important rôle in the preservation of food products by sodium benzoate and sulfurous acid (or its salts).

A study of papers on the toxicity of various reagents to microorganisms showed that previous attention had been given principally to their disinfecting power, that is, killing action, rather than to their

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preservative effect. However, Herter (1910) reported that 0.2 per cent of sodium benzoate retarded growth of and gas production by *Bacillus coli* in plain glucose broth, but had no noticeable effect in the same medium in the presence of calcium carbonate. He made no determination of hydrogen-ion concentration and gave the factor of acidity only passing attention. Barnard (1911) stated that benzoic acid is a more effective preservative than sodium benzoate but cited no experimental evidence to support his statement.

Held (1915) found that benzoic acid was more effective in a medium of low than in one of high protein content. He stated that when the protein was 'bound' by some other acid, such as tartaric, the concentration of sodium benzoate necessary for disinfection was lessened.

Perry and Beal (1920) found that 0.5 per cent of sodium benzoate was required to prevent the growth of the yeast *Saccharomyces cerevisiae* and 3.0 per cent was required to kill the cells. They also stated that benzoic acid was more effective than sodium benzoate in preventing growth of yeasts and molds.

Bonacorsi (1923) reported that the pH value of the medium greatly affected the killing action of several common disinfectants on microorganisms.

Fleischer and Amster (1922) reported that the disinfecting action of acid dyes was enhanced by a decrease in pH value and that of basic dyes by an increase in pH value.

Waterman and Kuiper (1925) stated that the inhibitive action of the salts of cinnamic, salicylic, and benzoic acids on *Penicillium glaucum* was much lower than that of the free acids.

Kuroda (1926) found that the disinfecting action of several phenols and aromatic acids on *Bacillus coli* and *B. prodigiosus* was much greater at pH values of 1.4 to 3.5 than at pH 5.0 to 8.9.

Cruess and Richert (1929) published a preliminary report of certain observations made upon the effect of pH value on the inhibiting action of sodium benzoate on the growth of several common food spoilage microorganisms; the inhibiting action was much greater at pH values below 4.5 than at 4.5 to 8.0.

Behre (1930) has summarized previously existing information in a recent article on food preservatives, including relation of pH value to preservative action.

The data obtained in our investigations are presented separately for each preservative and in the following order: sodium benzoate, sodium salicylate, sodium sulfite ('sulfurous acid'), 'acetic acid,' sodium chloride, and formaldehyde. These experiments were con-

ducted with pure cultures. However, in connection with this investigation tests of a practical nature were also made with mixed cultures and commercial food products such as olives, asparagus, avocado pulp, etc., in order to determine whether the results with pure cultures were applicable in commercial practice. The results of these experiments appeared in a technical journal article by Cruess (1931).

SODIUM BENZOATE

The microorganisms used with sodium benzoate were *Saccharomyces ellipsoideus* (strain 66) isolated from naturally fermenting California grape juice, one strain of *Mycoderma* isolated from cucumber pickle brine and another isolated from fermented apple juice, two species of *Penicillium*, a gray *Mucor* isolated from fresh fruit, a culture of lactic bacteria (E. B. Fred's culture No. 124–2), vinegar bacteria culture from cider vinegar, and cultures of *Bacillus coli, B. sporogenes, B. subtilis,* and *B. botulinus* from the Bacteriology Department of the University of California.

Concentrations of Sodium Benzoate to Prevent Growth in Liquid Media at Various pH Values.—For the vinegar bacteria and lactic acid bacteria, grape juice and apple juice were used; for *Bacillus coli, B. subtilis,* and *B. sporogenes,* a broth of the following composition was prepared: bacto-pepton, 10.0 grams; Libby's extract of beef, 10.0 grams; glucose, 7.5 grams; $MgSO_4$, 0.01 gram; KH_2PO_4, 0.25 gram; $(NH_4)_2HPO_4$, 0.25 gram; and water to make 1,000 cc. The three media were brought to various pH values by the addition of citric acid or sodium hydroxide. They were then subdivided into 100 cc portions to which were added amounts of benzoate ranging from none to an amount at each pH value which preliminary tests indicated to be sufficient to prevent growth. The liquids were then placed in plugged tubes and sterilized at the temperature and time required for the pH value concerned (as determined by preliminary trials); thus, liquids below pH 4.5 were heated one hour at 100° C, and those above pH 5.0, were sterilized by the usual intermittent three-period heating at 100° C. Since the pH values in some cases changed considerably during sterilization, the values reported in the tables are those taken after sterilization.

The buffering effect of the sodium benzoate is evident. In alkaline solutions the decrease in pH value is probably due to the formation of organic acids by the action of the alkali on the hexose sugars. The changes in pH value of liquids of high and of low pH value in the presence of the benzoate were, in most cases, greater than those in its absence. It tended to buffer to a pH value of 5.4–5.5.

TABLE 1

TYPICAL CHANGES IN pH VALUE DURING STERILIZATION OF CULTURE MEDIA

No benzoate added		Benzoate added in amounts shown		
pH value before sterilization	pH value after sterilization	Per cent benzoate added	pH value before sterilization	pH value after sterilization
3 2	3.6	0.05	3 2	3.6
4.0	3.9	0.15	4 0	4 0
4 4	4.5	0 15	4 4	4.6
5.6	5.0	1.50	5 6	5.4
6 0	5 7	1.50	6.0	5.5
7 4	7.3	1.50	7.4	6.1
9.0	8 6	1 50	9 0	7.4
10.0	8.7	1.50	10.0	7 9

Transfers of the various pure cultures, previously listed, were made to the sterilized tubes of media representing the various pH values and benzoate concentrations. The fermentation organisms used for inoculation were grown for five days in grape juice; the molds were grown on grape juice until abundant formation of spores had occurred; and the acid-intolerant organisms were grown in nutrient broth at 37° C for 5 days before transfer to the tubes of media containing benzoate. In all cases noninoculated tubes were retained for comparison with the inoculated ones in order to facilitate detection of growth. The tubes were stored at room temperature for six months. Regular observations were made to determine evidence of growth. In most positive tubes growth made its appearance in less than two weeks. All cultures in which growth was doubtful were examined microscopically.

The effect of pH value on the concentration of benzoate to prevent growth may be seen from the data presented in tables 1 and 2. Owing to the fact that not all of the experiments were made simultaneously nor with media of the same range of pH values, the pH values are given in the tables for each organism.

Similar experiments were conducted with *Bacillus botulinus* (*clostridium botulinum*). An asparagus juice medium was prepared, tubed, stratified with neutral oil, and sterilized. Tubes in duplicate of the sterile media of several pH values ranging from pH 4.0 to 8.6 were inoculated with spores of *B. botulinus* (*cl. botulinum*) grown in brain medium and detoxified by heat. The culture from which the spores were taken was rapidly fatal to guinea pigs before detoxification and nontoxic to them after heating to detoxify. The spores were still active,

TABLE 2

Effect of pH Value on the Concentration of Sodium Benzoate Necessary to Prevent the Growth of Acid-Tolerant Microorganisms

Saccharomyces ellipsoideus from grapes		*Mycoderma* from cider		*Mycoderma* from pickle brine		*Penicillium* (green)	
pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth
2.3	0.02	2.5	0.03	2.7	0.05	2.5	0.02
2.8	0.06	3.6	0.05	3.8	0.06	3.6	0.03
3.5	0.09	4.3	0.08	4.7	0.50	4.3	0.08
4.0	0.10	5.0	0.25	5.4	1.00	5.0	0.30
4.9	0.40	6.5	More than 1.5	7.3	4.00	6.5	1.50
5.2	0.45	......	....	.....	....	7.3	2.60
6.2	More than 1.5	......	......	10.0	0.70	8.5	More than 1.5
7.3	3.4	......	....	......	....	10.2	More than 1.5
9.5	More than 1.5	.....	....	......	....	11.0	0.90
Penicillium (gray)		*Mucor*		Vinegar bacteria		Lactic bacteria	
pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth
2.4	0.03	2.4	0.04	2.4	0.04	3.6	0.05
3.0	0.06	3.0	0.06	3.0	0.06	3.9	0.06
4.2	0.08	4.2	0.08	4.2	0.10	4.0	0.06
4.5	0.20	4.5	0.20	4.5	0.20	4.5	0.12
5.2	0.50	5.2	0.50	5.2	0.70	5.0	0.20
6.0	More than 1.5	6.0	1.20	6.0	More than 1.5	5.7	0.60
7.0	More than 1.5	......	......	7.0	More than 1.5	7.3	3.4
......	....	7.3	3.4	7.3	3.4	8.6	More than 1.5

TABLE 3

Effect of pH Value on the Concentration of Sodium Benzoate to Prevent Growth of Several Acid-Intolerant Microorganisms

pH value	*Bacillus coli*	*Bacillus subtilis*	*Bacillus sporogenes*
	Benzoate grams per 100 cc to prevent growth		
4.0	0.00	0.00	0.00
4.5	0.06	0.00	0.04
5.0	0.12	0.08	0.12
5.7	0.60	0.40	0.80
7.3	2.40	1.20	2.60
8.6	Growth at 1.5	Growth at 1.5	Growth at 1.5

however, after detoxifying by heat, for transfers from the detoxified suspension grew readily and produced toxin in brain medium.

At pH 8.6, 3.0 grams of sodium benzoate per 100 cc was required to prevent growth; at pH 7.4, 2.0 grams per 100 cc; at pH 5.2, 0.2 gram per 100 cc; at pH 4.7, 0.075 gram per 100 cc, and at pH 4.0, growth failed to occur even in the complete absence of benzoate.

At pH 7.4 with 0.8 gram of sodium benzoate per 100 cc the medium became fatally toxic to guinea pigs fed by mouth, whereas at pH 4.7 with 0.1 gram of benzoate per 100 cc the medium remained nontoxic and apparently free of growth. Owing to lack of facilities, the liquids in the other tubes were not tested in this manner. Growth was judged as positive or negative in these other tubes by macroscopical appearance, odor, and microscopical appearance of stained specimens.

Considering first the acid-tolerant organisms, it is evident that at pH values below 4.0 the benzoate exerts its greatest toxicity. At these values, in most instances, less than 0.1 gram of benzoate per 100 cc was required to prevent growth. Between pH 4 and pH 6 the concentration required to prevent growth increased rapidly, that is, the toxicity of the benzoate rapidly decreased over this pH range. The critical point appears to be in the neighborhood of pH 4.5, as there was a very sharp increase in the benzoate required to prevent growth when the pH value was increased from 4.5 to 5.0 or 5.2. From pH 5.0 to 7.3 the increase was less rapid, and tolerance appeared to approach a maximum between pH 7.3 and 10.0. The fact that less benzoate was required to prevent growth of the *Mycoderma* and *Penicillium* at pH 10.0 than at pH 7.3 would indicate that the maximum tolerance of these organisms for benzoate lies between these two pH values and that beyond this maximum the concentration required to prevent growth decreases. *Saccharomyces ellipsoideus* failed to grow at pH 10.0, even in the absence of benzoate.

The data clearly show that the toxicity of sodium benzoate to the yeasts, molds, vinegar bacteria, and lactic bacteria used in these experiments is greatly affected by the pH value of the medium.

Of the organisms studied, the *Mycoderma* appeared to be the most resistant to the benzoate at pH values on the acid side of neutrality.

Considering next the organisms intolerant of acid in table 3, it will be observed that they also were much less resistant to sodium benzoate at pH values of 5.0 or less than at pH values of 5.7 to 8.3. None of these organisms grew at pH values of 4.0 or less, even in the absence of benzoic acid.

Bacillus subtilis proved less resistant than *B. coli* and *B. sporogenes* to sodium benzoate, and all three cultures were less resistant than the

acid-tolerant organisms of table 2. However, it is possible that at pH 7.3 the relatively high concentration of sodium ion resulting from neutralization of the medium and from the added benzoate exerted a slight toxic effect separate from that of the benzoate ion (granting that the benzoate ion is toxic, a doubtful assumption). The evidence indicates that the undissociated benzoic acid is the toxic agent.

Considering the data of the two tables as a whole, the evidence is conclusive that the pH value very greatly affected the concentration of sodium benzoate to prevent growth. In order to give this statement greater emphasis, the average concentrations of sodium benzoate to prevent growth at several pH values have been calculated, making use of the data given for the organisms of table 2. (See table 4 and fig. 1.)

TABLE 4

AVERAGE VALUES OF SODIUM BENZOATE TO PREVENT GROWTH OF ACID-TOLERANT MICROORGANISMS AT SEVERAL pH VALUES

pH value	Benzoate, grams per 100 cc to prevent growth	Number of species of microorganisms represented
2 3–2.5	0.030	6
2.7–3.0	0.060	4
3.5–4.3	0.082	7
4.5–4.7	0 245	5
4.9–5.2	0.407	7
5.7–6.0	1.100	3
7.3–10 0	3 370	6
10.0–11 0	0.800	2

Only two cultures grew at pH values of 10.0–11.0, and it is of interest to note that the data indicate that less benzoate is required to prevent growth of these two organisms (a *Mycoderma* and a mold) than at or near neutrality. Evidently the OH ion reduces their vigor.

Relation of pH Value to the Inhibiting Action of Sodium Benzoate on the Multiplication of Yeast.—In experiments similar to those presented in table 5, it was noted that growth of various microorganisms was less abundant, for a given pH value, at high than at low concentrations of sodium benzoate. Apparently, also, the retarding effect was relatively less pronounced at pH values near neutrality than at those at or below pH 4.

In order to obtain an approximate numerical measure of the observed retarding effect of sodium benzoate, sterilized 100 cc portions of filtered apple juice of pH 3.0, 3.9, and 6.0 containing the concentrations of benzoate given in table 5 were inoculated with approximately 1,200,000 cells of *Saccharomyces ellipsoideus* per 100 cc. At intervals the number of cells per cc in each sample was determined

by counting under the dry high power of a microscope equipped with a calibrated net eye piece. The samples taken for counting were diluted to a definite volume with water and mounted in a hemocytometer. Three mounts were made of each specimen and the cells in 25 or more squares were counted for each mount.

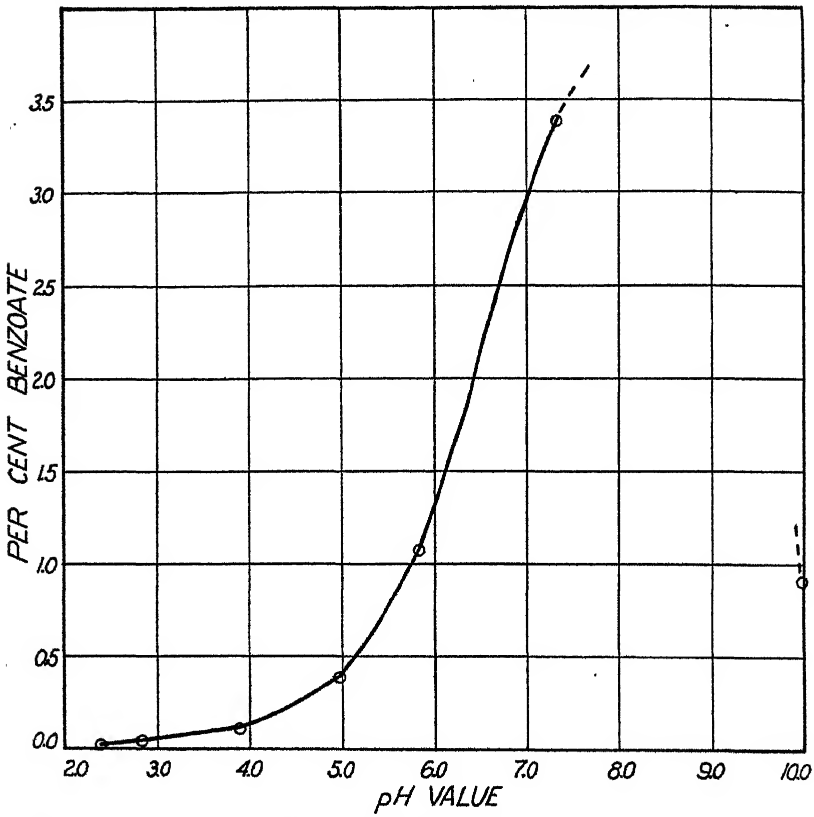

Fig. 1. The effect of pH value on sodium benzoate to prevent growth. Average for several acid-tolerant microorganisms. The solid line shows averages of data; the dotted line, the hypothetical curve from pH 7.3 to 10.0. The point for pH 10.0 is based on data for two organisms only, since most organisms failed to grow at this pH value, even in the absence of preservative.

The data show that the benzoate exerted a definite retarding effect on the multiplication of *Saccharomyces ellipsoideus,* except at pH 6.0 with 0.01 gram benzoate per 100 cc; in this case the benzoate appeared to exert little or no effect. At pH values of 3.0 and 3.9, however, 0.01 gram of benzoate per 100 cc greatly retarded multiplication. A concentration of 0.05 gram of benzoate per 100 cc permitted an increase in 6 days to 58,000,000 cells per cc at pH 6.0; an increase to only

4,800,000 at pH 3.9 and no increase whatsoever at pH 3.0. While counting bacterial cells by microscope is a difficult and rather inaccurate procedure, counting yeast cells in this manner gives reasonably consistent results. The differences reported in table 5 between the numbers of cells in samples of different pH values, but of the same

TABLE 5

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM BENZOATE ON THE MULTIPLICATION OF SACCHAROMYCES ELLIPSOIDEUS

pH value	Benzoate, grams per 100 cc	Number of cells per cc at 6 days	Number of cells per cc at 14 days
3 0	0.00	58,000,000	93,000,000
3.0	0.01	19,000,000	56,000,000
3 0	0 05	No growth	No growth
3.9	0.00	60,000,000	106,000,000
3.9	0 01	36,000,000	67,000,000
3.9	0 05	4,800,000	41,000,000
3 9	0.10	No growth	No growth
6 0	0.00	67,000,000	108,000,000
6 0	0.01	70,000,000	103,000,000
6 0	0 05	58,000,000	86,000,000
6.0	0.10	19,500,000	78,000,000
6.0	0.20	9,600,000	75,000,000
6.0	0 30	2,400,000	33,000,000
6.0	0.40	1,000,000	4,000,000
6.0	0 60	No growth	No growth

benzoate concentrations, are so great that there is no doubt concerning their general significance. Thus, the number of cells at the end of 6 days at pH 3.0 and 0.01 gram benzoate per 100 cc was 19,000,000 per cc; whereas at pH 6.0 with 0.01 gram benzoate per 100 cc it was 70,000,000. It is also evident that the benzoate at certain concentrations retards, but does not completely inhibit, multiplication of *S. ellipsoideus;* compare pH 6.0 and 0.01 or 0 grams benzoate per 100 cc with pH 6.0 and 0.4 gram benzoate per 100 cc. It is interesting to observe from table 5 that there is evidence of some stimulating effect of the benzoate at pH 6.0 and 0.01 gram of benzoate per 100 cc. However, the evidence of such an effect is considerably stronger in table 7. See discussion of this point following table 7.

Retarding Action of Sodium Benzoate on the Rate of Alcoholic Fermentation at Various pH Values.—In one experiment, 100 cc portions of sterile apple juice of pH values of 3.0, 3.9, and 6.0 and containing the concentrations of sodium benzoate indicated in table 6 were each inoculated with 1 cc of a vigorously fermenting culture of *Saccharomyces ellipsoideus,* strain 66. After three months' storage at room temperature the Brix degree of each sample was determined by means of an accurate hydrometer graduated to 1/10° Brix. Decrease in

Brix degree was taken as an indication of an increase in the extent of fermentation. Fermentation at this time had ceased in all cultures.

At pH 3.0 fermentation was completely inhibited by 0.05 gram of benzoate per 100 cc whereas at pH 6.0 some fermentation and active growth occurred at 0.6 gram of benzoate per 100 cc. Apparently, some growth also occurred at 0.8 gram of benzoate per 100 cc and pH 6.0. The benzoate was at least 16 times as toxic to *Saccharomyces ellipsoideus* at pH 3.0 as at pH 6.0, judged by its effect on fermentation.

TABLE 6

EFFECT OF pH VALUE OF APPLE JUICE ON FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

pH value	Sodium benzoate, grams per 100 cc	Brix degree average of duplicates	pH value	Sodium benzoate, grams per 100 cc	Brix degree average of duplicates
3.0	0.00	0 4	6.0	0.00	0.5
3.0	0 01	3.7	6.0	0.01	0 5
3.0	0 05	15 5	6.0	0 05	0 5
3 9	0.00	0.5	6.0	0 10	0.6
3.9	0.01	0 7	6.0	0.20	10.5
3.9	0.05	11.0	6.0	0.30	14.0
3.9	0.10	15.4*	6 0	0.40	12.2
3.9	0 20	15.5	6 0	0 60	13.0
Sterile check	0.00	15.5	6.0	0.80	15 0

* A decrease of less than 0.5° Brix is probably not significant owing to possible variation in the loss of moisture by evaporation from the individual cultures during the long incubation period.

The retarding action of sodium benzoate on the rate of fermentation was investigated further in several series of experiments by measuring the loss in weight of duplicate inoculated 100 cc samples of apple juice representing three pH values and several concentrations of sodium benzoate. The results from one such experiment are given in table 7, and several selected fermentation curves representing the three pH values are presented in figure 2.

It is seen from table 7 that a given concentration of sodium benzoate, for example, 0.02 gram per 100 cc, retarded the rate of fermentation much less at pH 4.5 than at pH 3.0. Likewise 0.1 gram of benzoate per 100 cc retarded fermentation considerably less at pH 7.0 than at 4.5. There was some stimulation of fermentation at pH 4.5 with 0.02 and 0.04 grams of benzoate per 100 cc and at pH 7.0 with 0.1 and 0.2 grams per 100 cc. This observation was in accord with the general principle that small concentrations of antiseptics stimulate the activity of microorganisms.

Four other series of fermentations were conducted with results similar to those reported in table 7. These definitely supported the

finding that at each pH value there exists a concentration below which the preservative stimulates and above which it retards yeast activity.

Growth of Penicillium Mold in 10 Per Cent Sodium Benzoate Solution.—In addition to the systematic experiments made to determine the relation of pH value to the toxicity of sodium benzoate to microorganisms, the following interesting chance observation was made:

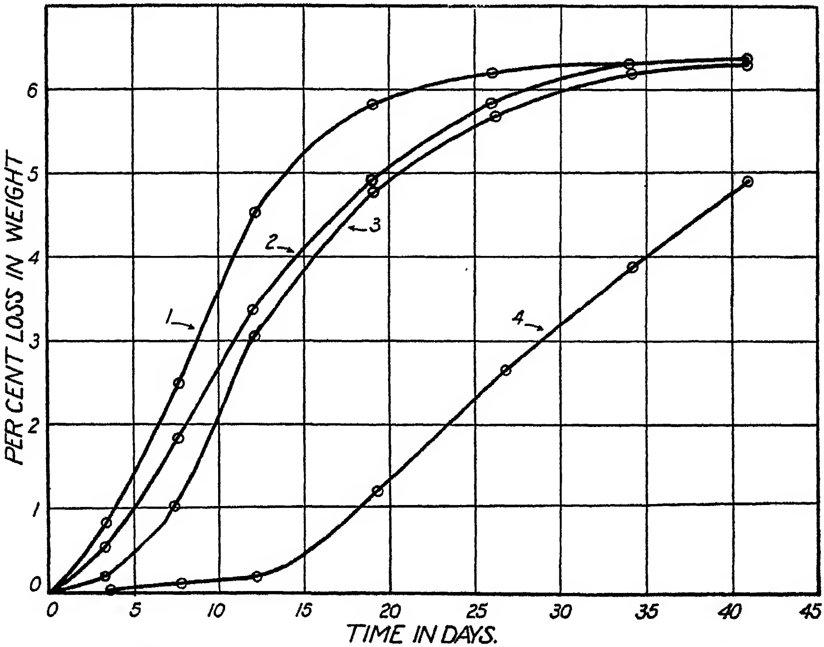

Fig. 2. Effect of pH value on the retarding action of sodium benzoate on yeast fermentation.

1. pH 4.5 and 0.0 grams per 100 cc
2. pH 7.0 and 0.2 gram per 100 cc
3. pH 4.5 and 0.06 gram per 100 cc
4. pH 3.0 and 0.02 gram per 100 cc

A liter bottle containing about 400 cc of 10 per cent sodium benzoate solution with a pH value of approximately 7.5, prepared in July, 1929, and stored at room temperature had developed a considerable quantity of cotton-like mycelial growth of a mold by November, 1930. By plating on nutrient agar agar the mold proved to be a strain of *Penicillium glaucum.* Spore formation was absent in the benzoate solution but was profuse on the agar plates.

This observation illustrates in a very striking manner the extremely low toxicity of sodium benzoate in solutions near neutrality.

Relation of pH Value and Benzoate to Preserve Several Food Products.—It was found that spoiling of cubed melon preserves, maraschino style grapes, ripe olives, avocado pulp, prune pulp, car-

TABLE 7

EFFECT OF pH VALUE ON THE RETARDING EFFECT OF SODIUM BENZOATE ON FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

Time in days	Benzoate, grams per 100 cc at pH 3.0		Benzoate, grams per 100 cc at pH 4.5						Benzoate, grams per 100 cc at pH 7.0							
	0	0.02	0	0.02	0.04	0.06	0.08	0.10	0	0 1	0 2	0.4	0 6	0 8	1.0	1 5
	Loss in weight in grams per 100 cc															
4	0.90	0.06	1 03	0.85	0 88	0.11	0.00	0.00	0 46	0 65	0 48	0 32	0 08	0.00	0 00	0.00
7	2.29	0.08	2.45	2 67	2.79	1.09	0.13	0.04	1.33	1 78	1 44	1 22	0.23	0.26	0.19	0.12
12	3.96	0.11	4.56	4.96	5.04	3.10	1.44	0.22	2.75	3.32	2.71	2 54	0 57	0 66	0.44	0 33
19	5.41	1.15	5 86	6.11	6.21	4.83	2.91	1 47	3.46	4.86	4.16	3.83	1.03	0 97	0 60	0 51
26	5.97	2 54	6.14	6.40	6 51	5.76	3.76	2 27	5.16	5.88	5 37	5 13	1 31	1.14	0 75	0.61
34	6.19	3.82	6.21	6.46	6 64	6.20	4.23	2.75	5.54	6.32	5 97	5.73	1 52	1 41	0.93	0 73
41	6 23	4.85	6.37	6 50	6.70	6.53	4.77	3.22	6.19	6 37	6 46	6 50	1 87	1.68	1.18	0 84
47	6.29	5.35	6.47	6.54	6 74	6.72	5.08	3 51	6.54	6 93	6 70	6.66	2.15	1 89	1.34	1 02
56	....	5.83	6.52	......	..	......	5.51	3.77	6 85	7 05	6.82	6 94	2.59	2.24	1 69	1 15
88	....	6.74	....	......	......	....	6.83	4.98	7 31	......	......	......	4 05	3.56	2.41	1.69

bonated beverages, asparagus, string beans, green peas, and artichokes could be prevented with 1/10 of 1 per cent or less of sodium benzoate when the pH value did not exceed 4.0. Near neutrality 2 per cent of sodium benzoate failed to prevent growth of molds, yeast, and bacteria. Therefore, the principle that the preservative action of sodium benzoate depends upon the pH value of the medium applies to foods as well as to the various culture media previously reported in this paper. Details of these experiments are given in a recent article by Cruess (1931).

OTHER PRESERVATIVES

The effect of pH value of the medium on the toxicity of sodium salicylate, sodium sulfite ('sulfurous acid'), 'acetic acid,' sodium chloride, and formaldehyde was studied in a manner similar to that already described for sodium benzoate. The first three preservatives were chosen because, like sodium benzoate, they represent weak acids or salts of such acids; sodium chloride was chosen because it represents the important class of neutral salts; and formaldehyde, because it represents a group of nondissociating organic compounds that should not be affected chemically by the pH values used in these tests. Formaldehyde, sodium salicylate, and salicylic acid are no longer permitted as food preservatives.

Sodium Salicylate.—Using the procedure outlined earlier in this report for sodium benzoate, the concentrations of C.P. sodium salicylate required to prevent growth of four different microorganisms were determined, with the results given in table 8.

TABLE 8

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM SALICYLATE REQUIRED TO PREVENT THE GROWTH OF MICROORGANISMS

pH value	*Saccharomyces ellipsoideus*	*Mucor* mold	*Penicillium* mold	Mixed culture of acetic bacteria
	Salicylate to prevent growth, grams per 100 cc			
2.5	0.02	0.02	0.04	0.02
3.5-3.8	0.06	0.15	0.10	0.08
7.0	3+	3+	3+	3+

Growth was positive in all cultures of pH 7.0 containing 3 grams of sodium salicylate per 100 cc.

As with sodium benzoate, a very much higher concentration (at least 150 times greater) of sodium salicylate was required to prevent the growth of common fermentation organisms at pH 7.0 than at pH 2.5. The toxicity of the salicylate at the three pH values used was similar to that previously observed for sodium benzoate.

Fermentation tests with cider containing sodium salicylate were conducted as previously described for sodium benzoate, with the results given in table 9. Data for several of the fermentation tests have been omitted to reduce the width of the table.

TABLE 9

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM SALICYLATE ON THE RATE OF FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

Time in days	pH 2.5		pH 3.5		pH 7.0		
	Salicylate concentration		Salicylate concentration		Salicylate concentration		
	0 gram per 100 cc	0.02 gram per 100 cc	0 gram per 100 cc	0.04 gram per 100 cc	0 gram per 100 cc	0.08 gram per 100 cc	2.0 grams per 100 cc
	Loss in weight in grams per 100 cc						
1	0 13	0 00	0.38	0 00	0.00	0 00	0.00
2	1 40	0 00	1 70	0 00	0 75	0 82	1.20
3	2 58	0 00	2 96	0 00	2 05	2.14	2 98
4	3 58	0 00	4 06	0 73	3.12	3 29	4 11
6	5 40	0 43	5.80	2 20	5 21	5.40	5.64
10	7.01	0 56	7.30	3.66	6 85	6 71	7.04
13	7.92	0 85	7 34	4 11	7 22	7.02	7.56
16	8.25	1.34	7 95	4.84	7.86	7.53	8 26
21	8.78	1.69	8.36	5 65	8 19	7.82	8.57
24	9 06	2.08	8.42	6.45	8 50	8 11	8 88
32	9 59	2.97	9.40	7.85	9 25	8 83	9.60
34	9 69	3 15	9 60	8.14	9.40	8 98	9.77
36	9 98	3.81	10.08	8 90	9.85	9.41	10 19

At pH 7.0 none of the concentrations of sodium salicylate used appreciably affected the rate of fermentation. At pH 2.5 and 3.5 beginning of fermentation was delayed by small concentrations of salicylate and the rate of fermentation was retarded.

Sodium Sulfite ('Sulfurous Acid').—In a manner similar to that described for sodium salicylate the effect of pH value on the toxicity of sodium sulfite to four different kinds of microorganisms was determined. The term 'SO_2' or 'sulfurous acid' is commonly used in expressing the concentration of sulfites in foods but when so used should be placed in quotation marks, since several compounds of sulfur are involved. While Na_2SO_3, sodium sulfite, was used as the source of 'SO_2', it is probable that at different pH values the sulfur exists in several different forms such as H_2SO_3 (undissociated), and as HSO_3 and SO_3 ions. From our results, it would seem that it is not the SO_3 ion that exerts the toxic action but more likely the undissociated H_2SO_3, or the HSO_3 ion, or possibly the anhydride, SO_2.

Inoculation, incubation, and observations were conducted as previously described for sodium salicylate. Table 10 gives the data obtained.

TABLE 10

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM SULFITE, EXPRESSED AS 'SO_2', REQUIRED TO PREVENT GROWTH OF MICROORGANISMS

pH value	*Saccharomyces ellipsoideus*		*Mucor* mold		*Penicillium* mold		Mixed bacteria	
	Initial 'SO_2' concentration to prevent growth*							
	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc
2.5	200	0.02	200	0.02	300	0.03	100	0.01
3.5	800	0.08	600	0.06	600	0.06	300	0.03
7.0	Above 5000	0.50	Above 5000	0.50	Above 5000	0.50	1000	0.10

* Parts per million multiplied by 0.0001 gives per cent: thus 200 p.p.m.=0.02 per cent.

The pH value of the medium exerted a very marked effect on the toxicity of the preservative. Thus, at pH 7.0 growth of three organisms occurred abundantly in the presence of 5,000 p.p.m., that is, 5,000 milligrams of 'SO_2' per liter (0.5 per cent). The bacteria, however, were less resistant and 1,000 p.p.m. prevented growth, even at pH 7.0.

The incubation period was 105 days, but observations were taken also at 3, 10, 63, and 79 days.

The concentrations given indicate the amounts of 'SO_2' added in the form of Na_2SO_3. Unquestionably, some 'SO_2' disappeared from the acidified samples as SO_2 gas and some of the SO_2 in all samples probably was oxidized to SO_3. Nevertheless, the results are qualitatively comparative and indicate that the preservative action of SO_2 is dependent upon the pH value of the medium.

Analyses of samples of pH 3.5 before and after sterilization gave the following results:

TABLE 11

LOSS OF 'SO_2' DURING STERILIZATION OF FRUIT JUICE

'SO_2' added as sulfite		'SO_2' after sterilization	
Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc
500	0.050	275	0.027
600	0.060	395	0.040
800	0.080	492	0.049
1000	0.100	672	0.067

The effect of pH value on the retarding action of 'SO_2' on fermentation by *Saccharomyces ellipsoideus* is shown in table 12.

TABLE 12

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM SULFITE, EXPRESSED AS 'SO_2' ON YEAST FERMENTATION

Time in days	pH 3.5				pH 7.0				
	'SO_2', parts per million				'SO_2', parts per million				
	0	100	200	300	0	100	300	1,000	5,000
	Loss in weight in grams per 100 cc								
1	1 2	1.2	0 0	0	0.6	0.6	1.2	1 0	0 0
2	6.6	2.4	0 0	0	6 6	6.0	6.8	5.4	1 8
3	9.0	6.0	0 0	0	12 0	1 2	11 4	11.4	2.4
6	13.8	13.2	1.8	0	13 2	13.2	13 2	13.2	9.6
8	15.6	15 6	10 8	0	15 0	15.0	15.0	15 0	12 0

As was true of the other weak acids the retarding action of 'SO_2' on fermentation was greater at the relatively low pH value of 3.5 than at neutrality, pH 7.0.

Potassium Acetate and 'Acetic Acid'.—Potassium acetate was used as a source of acetate radical and citric acid or postassium hydroxide to give the desired pH values. Sodium acetate proved unsuitable because of the toxicity of the Na ion at the higher concentrations of acetate. The growth of *Saccharomyces ellipsoideus, Penicillium* mold, *Mucor* mold and *S. cerevisiae* was prevented at pH 3.5 by 0.8 to 1.0 grams of *'acetic acid'* per 100 cc, whereas at pH 7.0, 4.0 grams of 'acetic acid' (that is, potassium acetate to give a concentration of acetate radical equal to that of 4.0 grams of acetic acid in 100 cc) failed to prevent growth. At pH 3.5 vinegar bacteria developed at all concentrations of 'acetic acid' used. This is not surprising, since *Bacterium aceti* converts ethyl alcohol to acetic acid and in commercial practice produces vinegars containing 10.0 grams of acetic acid per 100 cc. As with 'SO_2', evidently the undissociated acid, CH_3CO_2H and not the CH_3CO_2 ion is the toxic agent.

The effect of pH value on the retarding action of potassium acetate expressed as 'acetic acid' on fermentation is indicated in table 13.

At pH 7.0 the maximum concentration (4.0 grams per 100 cc) of 'acetic acid' failed to retard fermentation noticeably, whereas at pH 3.5 the rate was markedly retarded at 0.3 gram per 100 cc and failed to occur within the duration of the experiment at 0.6 gram per 100 cc. Evidently the undissociated HAc is the toxic agent, as at pH 3.5

TABLE 13

EFFECT OF pH VALUE ON THE RETARDING ACTION OF POTASSIUM ACETATE, EXPRESSED AS 'ACETIC ACID,' ON THE RATE OF FERMENTATION

Time in days	'Acetic acid,' concentration in per cent at pH 3.5		'Acetic acid,' concentration in per cent at pH 7.0				
	0	0.3	0	0.3	0.6	1.0	1.5
	Loss in weight in grams per 100 cc						
1	1.0	0.0	2.5	1.5	1 5	1.0	0 0
2	8.0	0 0	8 0	8.0	8 0	5.0	2.5
3	11.0	3.0	11.0	11.5	10 5	8 5	4 0
4	13.0	8.5	12.5	12.5	12.5	11.5	9.0
5	14.0	10 0	13 5	13 5	14 0	13.0	12 5
7	14 5	12.0	14 5	14 5	14 5	13.5	13.5
9	15 5	13.0	15.5	15.0	15.0	14.5	14.5

most of the acid is in this form and at pH 7.0 the acetate used is practically completely dissociated into Na and CH_3CO_2 ions. The H ion is naturally involved, but it alone at pH 3.5 does not prevent yeast growth, for fruit juices of pH 2.5 containing 10 times the H ion concentration of those of pH 3.5 were fermented readily in other experiments reported in this paper (citric acid being used to furnish the H ions). See for example table 15.

Sodium Chloride.—The concentrations of sodium chloride to prevent growth of two molds and a yeast were affected in some degree by the pH value of the medium as shown by the results given in table 14.

TABLE 14

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM CHLORIDE TO PREVENT GROWTH OF MICROORGANISMS

pH value	*S. ellipsoideus*	*Mucor* mold	*Penicillium* mold
	Approximate concentration of sodium chloride in per cent, to prevent growth		
2.5	14	16	18
3.5	20	20	20
7.0	20	20	20

The incubation period was approximately three months at room temperature. Apparently, the pH value of the medium is of much less consequence in the preservation of foods with sodium chloride than with the preservatives discussed previously in this paper. The

results agree well with those reported by Joslyn and Cruess (1929) in similar experiments with *Mycoderma* yeasts. The effect of pH value on the retarding action of sodium chloride on the rate of fermentation is given in table 15.

TABLE 15

THE EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM CHLORIDE ON FERMENTATION

Time in days	Per cent sodium chloride at pH 2.5			Per cent sodium chloride at pH 3.5			Per cent sodium chloride at pH 7.0		
	0	3	6	0	3	6	0	6	9
	Loss in weight in grams per 100 cc								
2	1 85	1 11	0 69	1 96	1.67	0.69	1 98	0.88	1.11
4	3 87	1 29	0 87	4.62	3.25	1.17	3 95	1.03	1.29
6	5.76	1.62	1.15	6.93	4 26	1 47	5.74	1 57	1.50
9	8.02	2.27	1 59	8 45	7 22	2 12	8.07	1 70	....
11	9 17	2.92	2 07	9.22	8.75	3 23	9 01	2.25	....
18	11.75	3.75	2 33	10 16	10 30	4 21	9.79	4.51	2 61
23	12.24	4.97	3 64	11.84	11.09	5.58	11 34	7.65	3 24
26	13.46	6 17	4.59	13 04	13.48	7.42	12.53	9.26	4.31
30	14 30	7.10	5.26	13.93	14.31	8 74	13.52	10 58	5.29

The sodium chloride retarded growth somewhat more at pH 2.5 and 3.5 than at 7.0. Citric acid was used to decrease the pH value to 2.5 and sodium hydroxide to increase it to 7.0; that neither of these substances exerted any significant retarding action can be seen by comparing the data in the three columns entitled "0 per cent sodium chloride."

Formaldehyde.—The effect of pH value on the concentration of formaldehyde to prevent growth of the four organisms was slight. The results of the experiment are given briefly as follows: growth at pH 2.5 was prevented by 0.015 gram formaldehyde per 100 cc but not by 0.0075 gram; growth at pH 3.5 and 7.0 was prevented by 0.03 gram formaldehyde per 100 cc but not by 0.015 gram. Therefore, at a very high acidity the formaldehyde is somewhat more toxic to microorganisms than at moderate acidity, or at neutrality. The effect of pH value, however, is very much less than that found for sodium benzoate, sodium salicylate, and potassium acetate, and resembles that obtained with sodium chloride.

Using the technique previously described, the effect of pH value on the retarding action of formaldehyde on fermentation was studied with the results given in table 16.

TABLE 16

EFFECT OF pH VALUE ON THE RETARDING ACTION OF FORMALDEHYDE ON FERMENTATION

Time in days	Formaldehyde, grams per 100 cc at pH 2.5			Formaldehyde, grams per 100 cc at pH 3.5		Formaldehyde, grams per 100 cc at pH 7.0	
	0	0.0037	0.0075	0.0037	0.0075	0.0075	0.015
	Loss in weight in grams per 100 cc						
1	1.20	1.66	0 15	2.34	1 14	1.13	0.60
3	4 47	5.36	0 40	6.20	2.32	3.93	3 72
6	6.53	7.94	3 78	8.21	6.96	6.57	4.46
10	7.16	9 24	6.95	8.75	7 83	8.10	5 33
15	7.44	10 11	7.56	9.13	8.44	8.72	5.77
20	7.61	10.73	7.84	9 43	8.90	9.20	6.08
24	7.78	11.23	8 05	9.67	9 26	9.58	6.34
27	8.03	11.60	8.42	10 22	....	10.14	6.74
31	8.21	11.85	8 67	10.34	....	10.56	7.03

Although the results are not so definite as those obtained with sodium chloride, they indicate that the preservative is somewhat more toxic at pH 2.5 and 3.5 than at 7.0. The difference is, however, far less pronounced than for sodium benzoate, salicylate, acetic acid, and sulfurous acid.

SUMMARY

The concentrations of sodium benzoate, sodium salicylate, potassium acetate ('acetic acid'), and sodium sulfite ('sulfurous acid') required to prevent the growth of yeasts, molds, and bacteria were much greater at pH 5 to 9.0 than at pH values in the distinctly acid range 2.0 to 4.5.

The effect of these preservatives on the rate of fermentation was modified in like manner by the pH value, but to a lesser degree.

The retarding effect of sodium benzoate on yeast multiplication was less at neutrality than at pH 3.0 and 3.9.

The concentrations of sodium chloride and of formaldehyde to prevent growth and the retarding action of these antiseptics on the rate of fermentation by *Saccharomyces ellipsoideus* were affected only moderately by the pH values used in these experiments.

The concentration of sodium benzoate required to preserve several food products was found to depend upon the pH value. In some instances, more than 200 times as much preservative was required at neutrality as at pH 3.0 or less.

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BODY SIZE AND METABOLISM

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INTRODUCTION

The statement that the basal metabolism of animals differing in size is nearly proportional to their respective body surfaces, is called the surface law.

Benedict has shown that this law is already over ninety years old, Robiquet and Tillaye having formulated it quite clearly in 1839. The history of the surface law is given in the paper of Harris and Benedict (1919). We may here only briefly mention the different ways in which it has been found. The early writers derived the law from theoretical considerations on a rather small experimental basis, as did Bergmann, who in 1847 had already written a book on the subject. Respiration trials were carried out by Regnault and Reiset, and Rameaux based the surface law on measurements of the amount of air respired per minute by two thousand human beings of different sizes. Rubner (1883) demonstrated the law in accurate respiration trials on dogs and Richet rediscovered it empirically on rabbits. The latter writes (p. 223): "C'est *après coup* seulement que je me suis avisé que la donnée *surface* était plus intéressante que la donnée *poids*."

Although Armsby, Fries, and Braman (1918, p. 55) found the surface law confirmed to a rather striking degree, this law is not at all so clear today as it appeared to its early discoverers. Carman and Mitchell (1926, p. 380) state the situation very well: "In spite of the theoretical weakness of the surface law, the computation of basal metabolism to the unit of the body surface seems at present the most satisfactory method available of equalizing experimental results for differences in the size of experimental animals."

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This is probably the point of view of most physiologists: they feel the necessity of having a method which allows the reduction of the metabolism of animals different in size to a common basis to make the results comparable for studies of other influences on the metabolism. The surface law offers such a common basis, but the theoretical weakness of this law is recognized.

It is obvious that the scientist should strive to overcome any theoretical weakness; that purpose is one of the essential stimuli for research. But, also, if the law between body size and metabolism were only considered as a means for equalizing results and estimating food requirements, it would still be important to get rid of the theoretical weakness of the method, because this weakness may mean a wrong application also.

Harris and Benedict (1919) based their critique of the surface law upon the classical investigation of the Carnegie Nutrition Laboratory on human metabolism. They separated the interspecific point of view from the intraspecific and came to the conclusion that within the human species there is no evidence of that law; DuBois (1927, p. 202) on the contrary, on the basis of the same experiments, finds the law confirmed.

The situation is therefore that the critique of the surface law based on material within the human species has not given definite results on the question of the validity of that law. Benedict himself approves of the application of the surface law for comparisons between species. Benedict and Ritzman (1927, p. 153) write: "The method of comparison is, however, justified on the basis of usage, provided a false significance is not attached to it and that a causal relation between body surface and heat production is not insisted upon."

In this paper the surface law, its theory and its application, is discussed mainly from the interspecific point of view. It may be claimed as a working hypothesis that there is a general influence of body size on the metabolism, an influence upon which the other influences on metabolism are superimposed. In order to study the general influence of size, animals as different in size as possible should be chosen so that this influence of size may predominate over the other influences.

EMPIRICAL RESULTS OF RECENT WORK ON METABOLISM

The surface law is illustrated by Voit's table (Voit, 1901, p. 120) which has received wide publication (Krogh, 1916, p. 142; Lusk, 1928, p. 123). From this table it follows that the basal metabolism of all animals is close to 1,000 Cals. per 24 hours per square meter of body surface. Recent determinations, however, show considerable deviation

TABLE 1

BASAL METABOLISM PER SQUARE METER OF BODY SURFACE AND PER UNIT OF POWERS OF BODY WEIGHT

Group No.	Animal	W Average weight, kilograms	Cals. per 24 hrs. per animal	Formula for surface area	Heat production in 24 hours in Calories per unit of: Body surface (sq. meter)	$W^{2/3}$	$W^{0.7}$	$W^{0.73}$	$W^{0.74}$	$W^{0.75}$	$W^{0.76}$	$W^{0.77}$	$W^{0.8}$	W
1	Steer	679	8,274	$0.1081 \times W^{5/8}$	1,300	107.1	86.3	71.0	66.3	62.2	58.3	54.6	44.8	12.2
2	Steer	342	6,255	$0.1081 \times W^{5/8}$	1,465	127.9	105.5	88.5	83.5	79.2	74.2	70.0	58.8	18.3
3	Cow	388	6,421	$0.1081 \times W^{5/8}$	1,387	120.7	99.1	82.8	77.9	73.6	69.2	65.2	53.2	16.5
4	Man	64.1	1,632	$71.84 \times W^{0.425} \times L^{0.725}$*	926	101.9	88.7	78.3	75.1	72.0	69.1	66.3	58.5	25.5
5	Woman	56.5	1,349	$71.84 \times W^{0.425} \times L^{0.725}$	848	91.6	80.1	71.0	68.2	65.5	63.6	61.1	53.5	23.9
6	Sheep	45.6	1,219.9	$0.124 \times W^{0.561}$	1,163	104.8	84.1	74.9	72.1	69.4	66.8	64.3	57.3	26.7
7	Male dog	15.5	525	$0.112 \times W^{2/3}$	776	84.5	77.2	70.8	69.1	67.2	65.4	63.6	58.5	33.8
8	Female dog	11.6	443	$0.112 \times W^{2/3}$	772	86.5	79.7	74.0	72.2	70.5	68.8	67.1	62.4	38.2
9	Hen	1.96	106	$5.86 \times W^{0.5} \times L^{0.6}$	676	67.7	66.2	65.0	64.2	63.8	63.6	63.1	61.8	54.1
10	Pigeon	0.300	30.8	$0.0985 \times W^{2/3}$	697	68.7	71.5	74.1	75.0	75.9	76.9	77.8	80.6	102.6
11	Male rat	0.226	25.5	$0.1136 \times W^{2/3}$	600	68.7	72.2	75.4	76.6	77.7	79.0	80.1	83.9	112.9
12	Female rat	0.173	20.2	$0.1136 \times W^{2/3}$	572	65.1	68.9	72.7	74.0	75.3	76.6	78.0	82.4	116.6
13	Ring dove	0.150	19.5	$0.0985 \times W^{2/3}$	701	69.1	73.6	77.9	79.4	80.9	82.5	84.0	88.9	130.0
	Average of all 13 groups, Calories:				914	89.6	81.0	75.1	73.3	71.8	70.3	68.9	65.0	54.7
	Average of 9 groups (excluding ruminants), Calories:				730	78.2	75.3	73.2	72.6	72.1	71.7	71.2	70.1	70.8
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	V, Coefficient of variability,† 13 groups, per cent:				±33.7	±23.9	±14.3	± 7.9	± 7.6	± 8.1	±10.0	±12.5	±21.5	±80.2
	V, Coefficient of variability, 9 groups, per cent:				±16.0	±16.9	± 9.1	± 5.6	± 6.5	± 8.2	±10.0	±12.1	±19.4	±61.9
	τ, Coefficient of tendency,‡ 13 groups, per cent:				+ 0.215	+ 0.163	+ 0.088	+ 0.024	+ 0.002	− 0.031	− 0.042	− 0.064	− 0.132	− 0.506
	τ, Coefficient of tendency, 9 groups, per cent:				+ 0.701	+ 0.808	+ 0.355	− 0.056	− 0.187	− 0.328	− 0.456	− 0.593	− 1.003	− 3.270

* L = Body length. † V = Standard deviation in per cent of the mean. ‡ τ = Term explained on p. 320.

Sources of data:

Group 1: *Benedict and Ritzman (1927)*: The value of 1,300 Cals. per square meter given in the summary (Moulton formula) was used, and the average weight of the two steers calculated from the table given by Benedict and Ritzman (p. 60).

Group 2: *Forbes, Kriss, and Braman (1927, p. 176, table 2)*: Average of 18 determinations on 4 steers, third to tenth day of fasting.

Group 3: *Forbes, Kriss, and Braman (1927)*: Average of 10 determinations on 4 cows, second to ninth day of fast.

Groups 4 and 5: *Harris and Benedict (1919, p. 57, 65, and 67)*: Average of 136 men and 103 women.

Group 6: *Ritzman and Benedict (1931, p. 31)*: Average of 7 determinations on 7 sheep lying 18 to 50 hours after food, 21° to 25° C.

Group 7: *Kunde and Steinhaus (1926, p. 129)*: Average of 10 determinations on 10 male dogs, 8.75 to 26.8 kilograms in weight, 18 to 20 hours after food.

Group 8: *Lusk and DuBois (1924, p. 213)*: Average of 16 determinations on 11 female dogs, 9.1 to 15.8 kilograms in weight.

Group 9: *Mitchell and Haines (1927, p. 932)*: Average of 44 determinations on 14 hens of 1.63 to 3.51 kilograms weight at 54° to 99° F in darkness.

Group 10: *Benedict and Riddle (1929, p. 523)*: Average of 3 determinations on 3 pigeons of pure race at 30° C; average of day and night runs.

Groups 11 and 12: *Mitchell and Carman (1926, p. 392)*: Average of results on 23 male and 18 female rats. Periods of rest only. 28° to 31° C.

Group 13: *Benedict and Riddle (1929, p. 523)*: Average of 21 determinations on 9 ring doves of pure race, at 30° C; average of day and night runs.

from this statement. The writer himself has found with an old rabbit a basal metabolism as low as 440 Cals. per 24 hours per square meter of body surface. Results of extensive work on basal metabolism which has been done in recent years in America are summarized in table 1.

The main objection to using a table such as this is that basal metabolism is not so well defined a term as might be desirable. As early as 1888, Hoesslin stated that there was no minimum metabolism of definite magnitude.

By observing certain rules, i.e., comparing animals under the same conditions, one may, however, obtain comparable results. The requirements to be observed are summarized by DuBois (1927).

It is difficult to tell exactly what the same conditions are for different animals: 24 hours after the last food, is for example, physiologically not the same for the steer as for the hen or the rat, also a certain environmental temperature may have a very different effect on a cow than on a pigeon.

Although it cannot be claimed that the results in table 1 have been obtained under the same conditions, there is nevertheless reason to believe that the animals compared in this table have all been studied in an environmental temperature above the so-called critical temperature, so that the metabolism is practically independent of variations in temperature. It must be admitted, however, that the question of the critical temperature is not entirely settled. The data in table 1 were obtained on mature individuals so that the influence of age should not be important. This statement may indeed still be open to some criticism. For example, it follows from a curve given by Benedict and Macleod (1929, p. 381), showing the influence of age on the heat production of female albino rats, that the rate of metabolism per square meter of body surface increases in these animals with increasing age, namely from 650 Cals. for rats of 8 months to 900 Cals. for rats which are 24 months old.[2] These data were obtained at an environmental temperature of 28.9° C. There is further reason to assume that in all cases summarized in table 1 the after-effect of food is excluded or at least does not seriously affect the result.

Differences in the degree of motility may have an influence on the figures of table 1 and may be partly responsible for the especially high rates of metabolism in ruminants compared with the other animals. The metabolism of the rats, for example, is taken only from the periods in which the rats were quiet; periods of activity were excluded. The influence of differences in motility cannot, however, change the general

[2] These authors calculated the surface area according to the Meeh formula: $S = 9.1 W^{2/3}$ (p. 361).

result; for Benedict and Ritzman (1927, p. 229) state that rarely more than 15 per cent difference in metabolism was found for the maximum difference in activity of their steers. The relatively low value of the hen may be in connection with the fact that the determinations had been made in darkness.

A rough comparison of the column giving Calories per unit of body surface with the column giving Calories per unit of W on the one hand and the column giving Calories per animal on the other may be taken as a confirmation of the opinion of Lusk and of Armsby: By calculating the rate of metabolism to the unit of body surface, one obtains much closer results than by calculating it to either the unit of body weight or to the whole animal as a unit.

The coefficient of variability in the calculation of the metabolism to the unit of body surface is ± 34 per cent. Although this coefficient is not even half of that resulting from the calculation to the unit of body weight, it seems at first that with such a variability one must deny the validity of the surface law as Benedict (1915, p. 277) has done.

A high coefficient of variability as such, however, is not sufficient reason to refute a suggested law. If the same deviations from the mean as those of the Calories per square meter in table 1 were so distributed among the different groups that the averages of six groups of the larger animals as well as the averages of six groups of the smaller animals would differ less than, say, 14 per cent $\left(\frac{34}{\sqrt{6}}\right)$ from the total average there would be reason to expect that with a material of six hundred instead of six groups on each side the difference of the means of each half from the total average might be within ± 1.4 per cent and that with increasing number of groups the average metabolism per square meter of large animals might be found more and more nearly the same as the corresponding average of small animals. If the deviations were so distributed there would be reason to expect that with increasing number of groups the surface law (the theory that the heat production per square meter of body surface is the same for large and small animals) could be proved with increasing accuracy and then the title of "law" would be justified in spite of the coefficient of variability of ± 34 per cent.

More serious for the surface law than the high coefficient of variability is the fact that the metabolism per square meter in table 1 shows a pronounced tendency to be increased with increasing size of the animal. If the results are grouped in two halves (omitting the middle group 7) six representing the larger and six the smaller animals the average heat production per square meter of the large animals is 512 Cals. or 56

per cent of the total average higher than the average for the small animals. In order to obtain a measure for the tendency of the metabolism to be increased with increasing body size the difference between the half averages in Calories has been divided by the corresponding difference in weight as shown in the following calculation:

Group No.	Average heat production per square meter $\overline{M}$	Difference $\Delta\overline{M}$	Average weight $\overline{W}$	Difference $\Delta\overline{W}$
	Cals.	*Cals.*	*kg*	*kg*
1– 6	1,182	512	262 5	260.1
8–13	670		2.4	

Thus $\frac{\Delta\overline{M}}{\Delta\overline{W}} = \frac{512}{260.1} = 1.97$ Cals. per sq. meter per kg.

The basal metabolism per square meter increases 1.97 Cals. per kilogram increase in body weight. As the average basal heat production is 914 Cals. per square meter, the increase per kilogram increase in body weight is 0.215 per cent of the mean. This is the coefficient of tendency τ in table 1.

The metabolism of the thirteen groups of animals has also been calculated to the unit of different powers of the body weight (W). The distribution of the deviations from the mean is best (τ is minimum) if the metabolism is calculated to the 0.74 power of the body weight. In this case the coefficient of variability is ± 7.6 per cent.

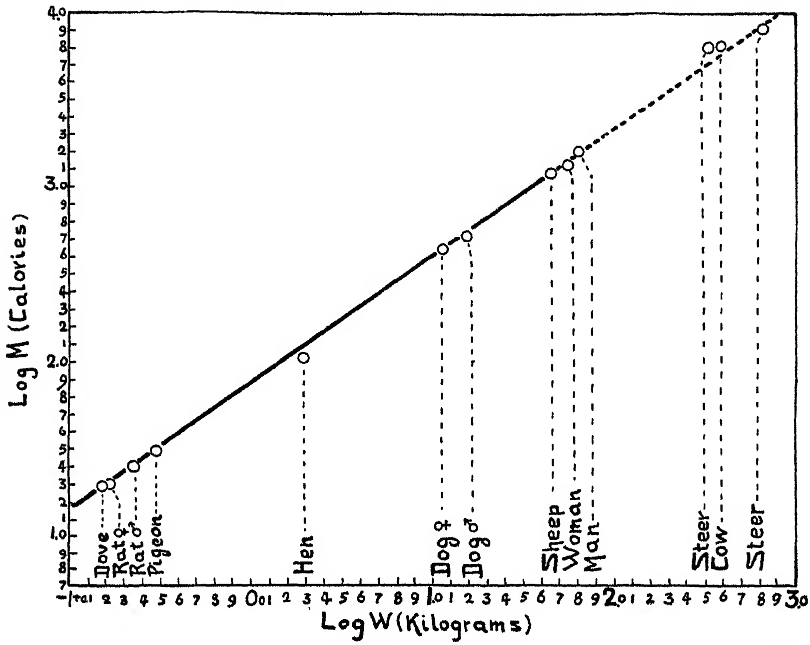
By excluding the ruminants from the calculation the deviation may be decreased. In this case the coefficient of variability is ± 16.0 per cent if the metabolism is calculated per square meter of body surface and as low as ± 5.6 per cent if the 0.73 power of the body weight is chosen as unit. If the different types of animals grouped together and the large range in body size are considered, it is surprising that any formula can be found which gives such a relatively low coefficient of variability.

A general formulation of the law expressing the relation between body size and metabolism may be found if the logarithm of the metabolism is plotted against the logarithm of the body weight; this has been done in figure 1. A straight line results, indicating that *the logarithm of the basal metabolism is proportional to the logarithm of the body weight.*

By differentiation of this function one finds that a small increase in metabolism per unit of the corresponding increase in body weight is proportional to the metabolism per unit of body weight:

$$\frac{dM}{dW} = K\frac{M}{W}$$

LOG. OF METABOLISM/LOG. OF BODYWEIGHT



It also may be expressed that the relative rate of increase of metabolism is proportional to the relative rate of increase in body weight:

$$\frac{dM}{M} = K\frac{dW}{W}$$

It follows from the linear function of the logarithms of metabolism and body weight that the metabolism per unit of a certain power of the body weight is constant. This, indeed, is no other result than was obtained by trying different calculations in table 1 and finding that the ¾ power of the body weight was the best-fitting unit.

It must be admitted that the material, though without doubt superior to that used heretofore as a basis for the surface law, is not yet homogenous and not adequate enough to decide conclusively to which power of the body weight (between the ⅔ and the ¾) the general influence of body size on the metabolism is most closely related. Two conclusions with regard to the surface law from the interspecific point of view may, however, be drawn:

1. The surface law is *confirmed* insofar as one gets closer results by calculating the basal metabolism to the unit of body surface than by calculating it to the unit of body weight.

2. The surface law is *refuted* insofar as the calculation of the metabolism to the unit of a power function of the body weight gives as close results as the calculation to the unit of body surface, or even closer.

THE THEORIES OF THE RELATION BETWEEN BODY SIZE AND METABOLISM

The question is now whether, on the basis of the material in table 1, the surface law should be abandoned and a weight-power law for the metabolism postulated, or whether there is reason to assume that the empirical result from table 1 is insignificant compared with the theoretical evidence of the surface law. To this end the amount of evidence for the statement that the metabolism is proportional to the body surface should be studied.

Four different theories which have been put forward to explain the surface law on physical or chemical bases may be distinguished, and then a biological explanation of the relation between body size and metabolism formulated.

Surface Law and Temperature Regulation.—The amount of heat required to maintain a constant temperature in a warm body surrounded by a cooler medium is proportional to the surface of that body. This has been, and still is designated in physiological papers, as the application of Newton's cooling law, although Harris and Benedict (1919, p. 135) have already criticized this terminology.

Newton's law of cooling may be written as follows:

$$\frac{du}{dt} = \frac{1}{k}(u_1 - u_2)$$

In a body with the temperature u_1 surrounded by a medium of the temperature u_2, the loss of temperature (du) per unit of time (dt) (rate of cooling) is proportional to the difference in temperature inside and outside. As the animal keeps the inside temperature constant, du becomes 0, and the law loses its application. There is no cooling, but heat flow.[3] The architect (Hütte, 1925, vol. 3, p. 335), in order to estimate the size of a furnace needed for a house, can calculate heat flow from inside to outside on the basis of Fourier's formula (Mach, 1919, p. 84):

[3] It may be mentioned that at Newton's time the two conceptions of temperature and heat were not kept clearly separated one from the other. (Mach, 1919, p. 132).

$$H = k \times O \frac{u_1 - u_2}{L} t$$

H = heat passed (calories)
k = coefficient of thermal conductivity
O = cross-section area of thermal conductor
L = length of thermal conductor
$u_1 - u_2$ = difference in temperature for the length L
t = time

This formula, originally derived for the flow of heat within a conductor may, as the application of the architect shows, be used for the calculation of the heat transmission entirely through a conductor.

For application to the problem of body metabolism, the surface area of an animal would be taken as the cross-section area and the thickness of the body covering as the length of the conductor.

The body covering of an animal includes the hair, the air in the interstices between the hair, the skin, the subcutaneous fat, and perhaps additional tissues (Benedict and Ritzman, 1927, p. 143; Benedict and Slack, 1911, p. 35).

The thermoconductive thickness, i.e., the thickness representing a certain average conductivity, of this cover is difficult to define. The situation may be simplified by introducing the term *specific insulation* of the animal and defining it as:

$$r = \frac{L}{k}$$

where r = specific insulation (resistance against heat flow)
L = the thermoconductive thickness of the cover
k = the average heat conductivity of the cover.

The following formula can then be derived:

$$\frac{H}{Ot} = \frac{u_1 - u_2}{r}$$

where $\frac{H}{Ot}$ = heat flow per unit of surface per unit of time (in the following tables given as small calories per square centimeter of body surface per day)
$u_1 - u_2$ = the difference in temperature inside and outside the covering, given in °C
r = the specific insulation

H means here the part of the total heat loss of the animal which passes through the skin. For an approximation, the total heat loss may be substituted for *H* and the additional amount resulting from heat

loss by other ways than the skin—especially the amount of heat given off through the respiratory organs—neglected. At abnormally high outside temperatures where the animal uses polypnoe as a means to prevent overheating the neglecting of the heat loss through the respiratory system might introduce a considerable error. The expression u_1-u_2 means the difference in temperature inside and outside of the animal's covering. For an approximation, u_2 may be taken as equal to the temperature of the environmental air. At high outside temperature, however, the temperature of the skin may be considerably lower than that of the surrounding air (because of evaporation of water and radiation). This fact, like that first mentioned, tends to decrease the reliability of the approximation for high outside temperatures.

The data in table 2 have been derived from my own earlier experiments.[4]

TABLE 2

SPECIFIC INSULATION OF RABBITS

Animal	Temperature, °C	u_1-u_2, °C	$\frac{H}{\overline{O}t}$	r
Old rabbit	18	22	49.7	0.44
	13	27	53.8	0.50
	4	36	72.7	0.50
Young rabbit	21	19	66.7	0.28
	13	27	74.4	0.36
	3	37	86.0	0.43

The specific insulation of the old rabbit remains fairly constant, but the young rabbit increases its insulation against heat loss with decreasing outside temperature. These results would seem to indicate that the young animal has a wider range of physical temperature regulation (regulation of blood circulation in the skin and the condition of fur).

Using data from Benedict and Ritzman (1927, p. 219) the calculations given in table 3 with regard to steers may be made:

TABLE 3

SPECIFIC INSULATION OF STEERS

No.	u_2, °C	u_1, °C	u_1-u_2, °C	$\frac{H}{\overline{O}t}$	r
1	2.9	37.7	34.8	174	0.200
	24.9	37.7	12.8	106	0.121
2	8.8	37.7	28.9	185	0.156
	28.3	37.7	9.4	119	0.079
3	3.4	37.7	24.3	173	0.198
	28.2	37.7	9.5	129	0.074
4	27.9	37.7	9.8	161	0.061
	7.3	37.7	30.4	145	0.210

[4] Carried out in the Swiss Institute for Animal Nutrition, Zurich.

The results show that steers can adapt their specific insulation considerably to the environmental temperature. In No. 4, where the steer had been first at high and then at low temperature, the regulation of the specific insulation was so pronounced that the animal had a reversed chemical regulation and produced less heat at low than at high environmental temperature.

Substantially the same results may be calculated from data on sheep published recently by Ritzman and Benedict (1931, p. 26, table 9).

TABLE 4
Specific Insulation of Sheep

No.	Temperature, °C			$\frac{H}{Ot}$	r
	Outside (u_2)	Body (u_1)	u_1-u_2		
1	3.4	39.2	35.8	129	0.277
	5.8	39.2	33.4	131	0.255
	23.3	39.2	15.9	153	0.104
2	8.7	39.4	30.5	109	0.280
	11.5	39.4	27.7	112	0.247
	27.5	39.4	11.7	117	0.100
3	3.2	39.4	36.0	131	0.275
	9.2	39.2	30.0	154	0.195
	30.7	39.2	18.5	172	0.049*
4	−0.1	39.2	39.3	121	0.325
	20.8	39.2	18.4	120	0.153

* Two days before lambing

The reversed chemical temperature regulation occurs in three of four cases in these experiments with sheep.

A behavior opposite to that of the one steer and the three sheep, namely a strict action of the chemical temperature regulation in Rubner's sense and even a reversed physical regulation may be calculated from data on fasting experiments with eight female albino rats published recently by Horst, Mendel, and Benedict (1930, tables 4 and 5). The calculation is presented in table 5.

TABLE 5
Specific Insulation of Rats

Day of fast	Activity	Temperature, °C			$\frac{H‡}{Ot}$	r
		Outside (u_2)	Body† (u_1)	u_1-u_2		
1*	15	16	37.5	21.5	126	0.171
1	16	26	37.5	11.5	66	0.174
7	28	16	37.5	21.5	123	0.175
7	10	26	37.5	11.5	50	0.230

* 22 hours without food.

† The body temperature, not found in the paper, has been supplied from direct measurements.

‡ The surface is calculated according to Meeh, $O = 9.1\,W^{2/3}$.

At the beginning of the fast the specific insulation of the rats at high and low environmental temperature was essentially the same. At the seventh day of fast the rats at high temperature had even a higher specific insulation than the rats at low outside temperature. The difference is such that it does not seem reasonable to explain it as within the errors of experiment or calculation, as, for example, due to the use of a constant body temperature. Some clue for an explanation may be found in the fact that activity was decreased during prolonged fasting at high outside temperature but was increased with prolonged fasting at the low outside temperature.

From earlier data of Benedict and Macleod (1929, p. 369, fig. 1), results on rats which confirm those obtained on steers, sheep, and rabbits may be obtained, as shown below:

Temperature, °C		$\frac{H}{Ot}$	r
Outside (u_2)	u_1-u_2		
10	27.5	180	0.153
28	9.5	88	0.108

That the animal can change its insulation has been clearly demonstrated by Hoesslin (1888, p. 329). He raised two dogs from the same litter, one at 32° C and the other at 5° C, and found from the different amounts of body substance produced by these two dogs, considering the amount of food consumed, that the one at 5° C had a metabolism only 12 per cent above that of its brother. Hoesslin states that if the heat loss had been the determining factor for the rate of metabolism (assuming a constant specific insulation), the difference in metabolism should have been several hundred per cent. The explanation was found in the fact that at the end of the 88 days of the trial the hair of the dog kept at 5°C weighed 129 grams, that of the other only 36 grams.

In a strict sense the surface law could be explained on the basis of Fourier's formula for the heat flow only if the specific insulation in small and large animals were the same. This situation cannot be expected, for it has just been shown that the insulation changes even in the same animal according to different outside conditions. It would not, however, be correct to discard the heat-loss theory entirely, as is often done.

The possibility of changing the specific insulation is actually limited. For example, steer *C* of Benedict and Ritzman (1927), which weighed 600 kilograms, had at an environmental temperature of 2.9° C a specific insulation of 0.200. If, for purposes of discussion the same heat conduc-

tivity is assumed for the body covering of the steer as has been found for the rabbit fur by Rubner (1895, p. 380), namely 6×10^{-5} calories per second, or 5 calories per 24 hours per square centimeter with a temperature gradient of 1° C per centimeter, the thermoconductive thickness[5] of the steer cover is found to be 1 cm. (According to the definition of the specific insulation given on page 323, it follows: $L = rk = 0.2 \times 5 = 1.0$.)

A mouse of 60 grams with the same heat production per unit of body weight and the same heat conductivity of the cover would require a thermoconductive thickness of covering of no less than 20 cm to keep its body temperature at the same level above the outside temperature as does the steer.[6] The fact is that the mouse produces 20 times as much heat per gram of body weight as does the steer, and animals of the size of a mouse would not be able to live as warm-blooded animals in the temperate and cold zones of the world if they had only the same rate of heat production per unit of body weight as a steer.

The heat-loss theory of the surface law is thus reasonable if one compares animals very different in size which are living at relatively low temperatures.

The heat-loss theory loses its application for explaining the surface law in animals which are living in warm climates where they have to operate regulating systems to get rid of a surplus of heat. The ability to give off heat and prevent overheating was, however, also related to the surface law by Rubner in 1902 (Lehmann, 1926, p. 575). The same statement can be made for the overheating theory as for the heat-loss theory, namely, that it does not apply to animals of similar size, but is reasonable if the animals compared differ considerably in size.

The sailors whom Robert Mayer had to bleed on board the ship "Java" in the Bay of Surabaya in the summer of 1842 had light red venous blood, a fact which led that young genius to the discovery of the law of conservation of energy. The blood was light red because the sailors had decreased their muscular activity in the hot zone in

[5] Defined on p. 323.

[6] The surface per unit of body weight, which in an animal is practically the same as the surface per unit of body volume, or the specific surface, is $\frac{W^{2/3}}{W} = W^{-1/3}$. The ratio of the specific surfaces of mouse to steer is thus the cube root of the inverse ratio of their respective body weights $\sqrt[3]{\frac{600 \times 10^3}{60}} = 10\sqrt[3]{10} = 21.6$. The surface per gram of mouse is therefore 20 times as large as the surface per gram of steer. With the same heat production per gram of body weight, the heat flow through 1 sq. cm of surface of a mouse should therefore be only 1/20 of that through 1 sq. cm of surface of a steer; consequently the specific insulation of the mouse should be 20 times as high as that of the steer.

order to prevent overheating. What would they have done with a heat production ten times as great, which per unit of body weight would correspond to the metabolism of a mouse? If animals varying much in size and living in hot regions are considered, the overheating theory of the surface law is thus acceptable.

For hot as well as for cold climates, therefore, the maintenance of a constant body temperature gives us a sound explanation for the surface law if animals of considerably different size are compared; this is an explanation only in the sense, however, that the regulation of body temperature is not the cause, but one of the conditions which influence the metabolism and is therefore a criterion, among others, in the selection of the fittest.

Surface Law and Nutritive Surfaces.—Puettner (Lehmann, 1926, p. 577), using older ideas such as those of Hoesslin, has stated that the surfaces of the intestinal tract and of the lungs and, finally, the surfaces of the individual cells of the animal are the important factors for the rate of metabolism, and that one may explain the surface law as resulting from the rate of diffusion of the nutrients through these internal surfaces.

Pfaundler (1921, p. 273) states correctly that the surfaces of the cells could be responsible for the surface law only if the cells in an animal merely grew but did not increase in number, because only in this case could the sum of the cell surfaces in an animal be proportional to its body surface. Pfaundler himself, however, attempts to explain the surface law basing his explanation on Buetschli's theory of the structure of the protoplasm, the "Wabenstruktur" (honeycomb structure). Pfaundler apparently believes that the sum of the surfaces of those hypothetical structures of the living substance in an animal should be proportional to the $\frac{2}{3}$ power of the body weight. This would imply that the protoplasmic elements of a man in linear dimensions should be ten times as large as the corresponding elements of the protoplasm of a mouse; or that one kilogram of protoplasm of an ox should contain the same number of protoplasm units as one gram of guinea pig plasm. It is doubtful whether any real basis can be found for such a logical consequence of Pfaundler's theory.

The final refutation of all attempts to explain the surface law with cell and cell-structure surfaces comes as a result of the modern research on the respiration of tissues; according to Terroine and Roche (1925), *homologous tissues of different animals have in vitro the same intensity of respiration.*

In the same year Grafe (1925) states: "The living protoplasma of the warm-blooded animals and maybe even of many cold-blooded

animals, shows as far as the respiration is concerned a certain uniformity and gets its specificity only by means of the influence of the regulating system of the animal."

Grafe, Reinwein, and Singer (1925, p. 109) found some differences in the respiration of tissues of different animals in vitro. The average oxygen consumption per gram of dry matter per minute is 0.2 cc for mouse tissue and 0.119 cc for that of the ox. These authors state, however, that this difference cannot explain the fact that in vivo one gram of mouse body uses up per unit of time 33 times as much oxygen as one gram of ox body.

The law of body size and metabolism is therefore not a matter of the tissues, but a matter of the organism as a whole.

TABLE 6

Blood Volume and Body Weight

Animal	Sources of formulas	Body weight, grams (W)	Blood volume, cc	Blood quantity, in per cent of body weight
1	*2*	*3*	*4*	*5*
Rabbit	Average of 22 determinations, table 1, p. 138	670–3,250	0.632 $W^{2/3}$	4.92
Guinea pig	Average of 9 determinations, table 16, p. 152	215–825	0.189 $W^{2/3}$	4.10
Mouse	Average of 10 determinations, table 20, p. 154	11.9–29.3	0.149 $W^{2/3}$	5.77

Surface Law and Composition of the Body.—Benedict has shown (1915, p. 298) that the proportion of inert body fat and *active protoplasmic tissue* influences the metabolism. This influence may be as effective as that of size within the human species. An influence of this kind cannot, however, be used as an explanation for the surface law if animals of considerably different size are compared. Thus Carman and Mitchell (1926, p. 380) have calculated that if a rat consisted entirely of active protoplasm, then a man, with his lower metabolism per unit of weight, should on that basis contain only 9.4 kg of active protoplasm.

Dreyer, Ray, and Walker (1910, p. 158) suggested that the *blood volume* of an animal was proportional to the surface area of that animal and that "the practice of expressing the blood volume as a percentage of the body weight is both erroneous and misleading." The results of these last named investigators may be summarized in table 6.

Column 4 of table 6 shows that according to the formulas of Dreyer, Ray, and Walker the blood volume is to be calculated by multiplying the ⅔ power of the body weight by a factor which varies directly with

the size of the animals, if different species are concerned. The blood volume per unit of $W^{2/3}$ in the rabbit is 4.2 times $\left(\frac{0.632}{0.149}\right)$ as large as that of the mouse. From column 5, on the other hand, it may be concluded that the blood volume per gram of body weight is not related to the size of the animals, i.e., that the blood volume is proportional to the body weight.

The theory of Dreyer, Ray, and Walker that the blood volume is proportional to the body surface (or the ⅔ power of the body weight) must therefore be refuted on the basis of their own results, at least from the interspecific point of view.

Recently Brody, Comfort, and Matthews (1928, p. 33) as a result of extensive research and ingenious calculation,[7] have claimed that "the weight of the kidney, the weight of the liver, and practically the weight of the lung, blood, stomach, and intestine increase directly with the body weight at the same relative rate as does the surface.'' Their results (see their fig. 6, p. 17) indicate, however, that the surface area follows the function $W^{0.71}$ and the blood volume the function $W^{0.83}$.

If animals of very different size are compared, it can be seen that the blood volume cannot be proportional to the body surface, but must be related to a function which is not far from the first power of the weight.

It may be that the differences in the blood quantity per unit of body weight in any one species are affected by age and fat content. Possibly the heavier animals used are on the average older and fatter. This idea gains strength from the work of Trowbridge, Moulton, and Haig (1915, p. 16), who state in relation to cattle that "the fatter the animal the smaller the proportion of blood."

Lindhard (1926, p. 669) found the blood quantity of man (11 healthy subjects) to be 4.9 per cent of the body weight. If the blood quantity were proportional to the body surface, the 70-gram body of the rat should contain 34 cc of blood, or 49 per cent.[8]

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[7] Surface integrator measurements on 482 dairy cows, 341 beef cattle, 11 horses, and 16 swine.

[8] If W_m be the weight of man and W_r the weight of rat we may formulate:

$$\text{Blood volume of man per } W^{2/3} \text{ unit} = \frac{0.049\,W_m}{W_m^{2/3}}$$

$$\text{Blood volume of rat per } W^{2/3} \textit{ unit} = \frac{xW_r}{W_r^{2/3}}$$

If the blood volume were proportional to $W^{2/3}$, the two quotients would be equal, thus:

$$x = \frac{0.049\,W_m W_r^{2/3}}{W_m^{2/3} W_r} = 0.049\left(\frac{W_m}{W_r}\right)^{1/3} = 0.049 \times 1{,}000^{1/3} = 0.49 = 49 \text{ per cent.}$$

It follows thus that the surface law is not a matter of the tissues or cells and cannot be a matter of the chemical composition of the animal, but is a matter of the animal as a whole. The two great regulators, the nervous and endocrine systems, control the intensity of blood flow and the distribution of the blood to the tissues, so that the respiratory metabolism of animals of different size is approximately proportional to the ⅔ power of the body weight.

Surface Law and Blood Circulation.—Loewy (1926, p. 22) has summarized data on the oxygen content of arterial and venous blood. It follows from his table that a liter of blood which passes the capillary system leaves on the average 60 to 70 cc of oxygen in the tissues, and further that this amount is independent of the size of the animal. It is therefore sound to assume that the amount of oxygen carried to the tissues per unit of time (intensity of oxygen flow) is on the average proportional to the amount of blood passing the tissues per unit of time (intensity of blood flow).

Hoesslin (1888) attempted to show that for geometrical and mechanical reasons the amount of blood carried to the tissues per unit of time must be proportional to the ⅔ power of the body weight. He bases his reasoning on the assumption of the geometrical similarity of large and small animals. This geometrical similarity means that all dimensions which are in certain arithmetical ratios in small animals are in the same ratio in large animals. Thus, if the cross-section area of the aorta of a small animal be *a* per cent of the cross-section area of the body or *b* per unit of the ⅔ power of the body weight, the aorta of a large animal also will have a cross-section area which is *a* per cent of the cross-section area of its body or *b* per unit of the ⅔ power of the body weight. This assumption, especially with regard to the aorta, has really been fairly closely confirmed by measurements of Dreyer, Ray, and Walker (1912), who found that the cross-section area of the aorta is proportional to a function of the 0.70 to 0.72 power of the body weight.

The amount of blood passing a certain cross section of the body per unit of time is the product of the sum of the cross-section areas of all blood vessels in that body cross section and the linear velocity of the blood flow. The linear velocity is, according to Volkmann (Hoesslin, 1888, p. 324), independent of the size of the animal. Therefore, concludes Hoesslin, the product, the intensity of blood flow, is proportional to the sum of the cross-section areas of the blood vessels and is thus proportional to the ⅔ power of the body weight, a suggestion which explains, according to him, also the fact that the metabolism is proportional to that power of the body weight.

As the capillaries of a horse are not ten times as wide as those of a guinea pig, but are of approximately the same size, it follows that the principle of similarity mentioned above applies only to the large vessels. Hoesslin's explanation of the surface law is therefore satisfactory only if we can understand why the linear velocity in the large vessels is independent of the body size.

The question may be related to the economy in energy consumption for blood circulation. The specific current energy, i.e., the energy necessary for the transport of 1 cc of blood through a given part of the duct, is higher for turbulent than for laminar flow, as has been stated by Hess (1927, p. 901). The same author demonstrated that under normal conditions the blood flows laminarily (1917, p. 477).

In certain pathological cases where the viscosity of blood is abnormally low, murmurings in the large vessels may be heard, which, according to Hess (1927*a*, p. 913) indicate that the normal velocity of blood flow cannot be far from the critical velocity, beyond which the flow would be turbulent.

According to Reynold (Hess, 1927, p. 900) the critical velocity is inversely proportional to the diameter of the duct.[9] If it were advantageous for the animal to maintain in its large vessels a velocity close to the critical, and if this advantage were the determining factor for the velocity of blood flow, one would expect, according to Reynold's formula, that the linear velocity of blood flow in animals of different size would be inversely proportional to the linear dimensions of the body or to the ⅓ power of the body weight. This expectation is in contradiction to the constancy of the linear velocity of blood flow, instead of being an explanation for it.

Hoesslin's theory of the relation between surface law and blood circulation is thus less satisfactory than it might appear at a first glance (see for example Lehmann, 1926, p. 577).

For a schematical comparison of the blood circulation in small and large animals three groups of vessels should be distinguished:

1. The larger arteries and veins, which may be called the individual vessels. They are dependent in size (diameter and length) upon the body size of the animal. Their number is independent of the size of the animal.

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[9] Reynold's equation for the critical velocity reads as follows:

$$v = \frac{2000\eta}{2rs}$$

v = critical velocity
η = viscosity of the fluid
s = density of the fluid
r = radius of the duct

2. A second group of vessels, represented by the capillaries, which may be termed the tissue vessels. Their size is independent of the size of the animal, but their number depends upon the amount of tissues and therefore upon the size of the animal.

3. The connecting vessels, which connect the system of the individual vessels with the capillary net work. The vessels of this group depend in size as well as in number upon the body size of the animal.

The amount of blood passing a cross section of the duct per unit of time is, for laminar flow, according to Poisseuille[10] proportional to the difference in pressure at the end of a given part of that duct and inversely proportional to the hemodynamic resistance. The hemodynamic resistance is proportional to the length and inversely proportional to the square of the cross section of the duct.

For the individual vessels, which may collectively be represented as a single vessel, the length is proportional to the $W^{1/3}$ and the cross section proportional to $W^{2/3}$. The hemodynamic resistance of this system is therefore proportional to $\frac{W^{1/3}}{W^{4/3}}$ or $\frac{1}{W}$.

The arterial blood pressure of animals is independent of the body size (Tigerstedt, 1921, p. 209). This may be expected from Hoesslin's point of view of the similarity of large and small animals, for it is a technical rule that pipes of different width in which the wall thickness is proportional to the diameter can stand the same pressure. (Hütte, 1925, vol. 1, p. 675.) If, however, in pursuance of this idea, it is assumed that there is the same difference in blood pressure for corresponding parts of the individual vessels of large and small animals, then according to Poisseuille's law the intensity of blood flow would be proportional to the body weight instead of being proportional to the ⅔ power of this term.

The same result is obtained for the tissue vessels if it is assumed that the number of available capillaries is proportional to the amount of tissue, and hence to the body weight, and that the average length and width of each capillary are independent of the body size. It is difficult, if not impossible, to verify this assumption. The number of open (but

[10] The law of Poisseuille may be formulated as follows:

$$V = \frac{q^2}{8\pi\eta L}\Delta P \times t \text{ where:}$$

V = volume of liquid passing a certain part of the duct
q = cross section of duct
L = length of duct
ΔP = difference in pressure
t = time
π = 3.14 . . .
η = viscosity

not the number of available) capillaries which are counted under the microscope varies according to whether the muscle from which a part is observed has been in action or at rest before the animal was killed.

Krogh (1929, p. 63) counted in a section from a stimulated muscle of the frog 195 open capillaries per square millimeter, while the corresponding unstimulated muscle had not more than 5.

Krogh (1929, p. 30) found on the average fewer open capillaries per unit of cross section in tissues of a large animal than in those of a small one; the muscle of a horse (550 kg) had 1,400 capillaries per sq. mm, and the muscle of a dog (5 kg) had 2,600 capillaries per sq. mm. Terroine (1924) bases his theory of the relation between body size and metabolism upon this fact. The average number of open capillaries is, however, a result of the regulation of blood flow by the nervous and the endocrine systems and cannot therefore be used as an explanation for the regulation of blood flow to a certain level.

Less contradiction is to be found if the surface law is related to the rate of *heart beat*. The total blood volume in an animal is proportional to the body weight (see p. 330), and the blood volume moved by one heart beat is, in mammals, a constant part of the total blood volume, namely 1/26 to 1/29, according to Vierordt (cited by Kisch, 1927, p. 1218). The pulse rate in the mouse (*Mus musculus*) is 520 to 780 beats per minute, in man 76, and in the horse 34 to 50. A frequency of 300 to 400 would be classed as extreme tachycardia in man (Winterberg, 1927, p. 671). The contraction of the heart muscle in the horse requires 0.1 second (Tigerstedt, 1921, p. 209); the pulse rate of the mouse would mean tetanus in the heart of a horse. These facts indicate why the pulse rate should be inversely proportional to a function of the body weight in animals of widely different weights, but they give no satisfactory clue as to why this relation should obtain exactly between animals of closely similar size. The situation is similar to that between the surface law and temperature regulation (see p. 326).

The pulse rate reported for different individuals of the same species differs so considerably that it would seem at first glance almost impossible to determine an exact relation between pulse rate and body size. For an approximate estimate, however, the logarithmic method as used by Brody, Comfort, and Mathews (1928) may be applied on data for the pulse rate of elephant, horse, cattle, sheep, and rabbit given by Rihl (1927) and the relation of pulse rate and body weight reduced to the equation:

$$P = 186 \times W^{-1/4}$$

where P = pulse rate (beats per minute)
W = body weight in kilograms

In order to give an explanation for the surface law, the pulse rate should be proportional to the $-1/3$ power of the weight instead of the $-1/4$ power.

If the volume per heart beat were exactly proportional to the body weight and the pulse rate were exactly proportional to the $-1/4$ power of the body weight, the intensity of blood flow would be proportional to the $3/4$ power of the body weight. This condition would really correspond to the empirical result on basal metabolism shown in table 1 (p. 317) more than to the surface law.

The influence of body size on metabolism may reasonably be related to oxygen transport, but no evidence can be found from these theoretical considerations that the metabolism of animals is more closely related to their geometric surface than to some other function, as for example the $3/4$ power of the body weight.

Biological Explanation of the Relation Between Body Size and Metabolism.—From the interspecific point of view, two of the four kinds of explanations for the influence of body size on metabolism stand criticism: regulation of a constant body temperature, and geometric and dynamic relations of oxygen transport. But neither the outside temperature alone nor the intensity of blood flow determines the metabolism. Lehmann (1926, p. 577) writes that the metabolism of an organ is not increased if it gets more oxygen, but that more blood is brought to the organ if it requires more oxygen. This teleological statement, however, is not an explanation either.

The biological theory is that those animals are the fittest in natural selection in which the metabolism is so regulated that the requirements for maintaining a constant body temperature and the energy requirements for the necessary mechanical work are in an economical relation with the geometric and dynamic possibilities of oxygen transport.

In the introduction, I claimed as a working hypothesis that there was a general influence of body size on metabolism, leaving the question open as to how this influence might be formulated. Neither the empirical results from table 1 (p. 317) nor the discussion of the theory of the surface law gave evidence for the belief that the rate of metabolism is more closely related to the body surface than to some other function of the body size. The general formulation of the law of body size and metabolism is that the logarithm of the metabolism is proportional to the logarithm of body weight.

Deduction.—The reason for the excursion into the theory of the surface law was the discrepancy between the surface law and the empirical results in table 1, based on the recent work on metabolism. The study of this theory fails to show that there is any evidence for a

closer relation of metabolism to the geometrical surface of animals than to some function of the body weight; for example, the ¾ power, which is in better agreement with the empirical results in table 1 (including ruminants).

APPLICATION OF RESULTS

The Unit of Body Size for Measuring the Relative Rate of Metabolism.—It follows from the result of metabolism studies as well as from the discussion of the theory of the surface law that metabolism can be related to a power function of the weight, and the unit of body surface given up. There are two reasons for hesitating to do so. First, the best-fitting power function cannot yet be given definitely. Further investigation may show that some unit other than $W^{3/4}$ may be preferable. Secondly, the unit of body surface has been relatively long in use, and much work has been done to develop it. Even if the theoretical and empirical weakness of the surface law is admitted, it may be preferable to keep the square meter of body surface as a unit of measurement as long as it proves to be useful, and especially if it meets the first requirement of any unit for measurement, namely, to be well defined. It seems, however, that the more work done to determine the surface area, the less is one able to define the unit of it for the measurement of metabolism.

The simplest method of determining the surface area of an animal was probably that of Richet (1889, p. 221). He calculated the surface from the body weight assuming the animals to be spheres. If a specific gravity of 1.0 is considered, the calculation of Richet would be:

$$S = 4.84 \times W^{2/3}$$

where S = surface in square centimeters
W = body weight in grams

Meeh attempted to get a closer approximation of the true surface of the animal by choosing different parameters of the ⅔ power of the weight instead of the sphere-constant 4.84. Meeh writes:

$$S = k \times W^{2/3}$$

where S = surface in square centimeters
W = weight in grams

and where k varies according to the different species of animals and seemingly even within one species; in man for example from 9 to 13, as Harris and Benedict (1919, p. 142) show in their history of the development of the unit of body surface. A table of the different Meeh factors is given by Lusk (1928, p. 123).

Later on, not only were different coefficients suggested, but also the exponents of the power function were varied. In addition ingenious methods have been developed to measure the surface area directly.

The natural question as to which of the different methods of determining the surface area gives the closest results for the true surface leads to a serious difficulty. What does belong to the true surface and what does not belong to it? In trying to answer this question one finds that not only the skin is elastic[11] but also the conception of its geometrical surface area on the living animal, and that fact, for this particular question, is worse. But suppose it would be possible to define exactly a true surface geometrically and to confirm what is indeed to be expected —namely, that the elaborate modern methods would allow us to determine the true surface area with a higher degree of accuracy than Richet's formula—the second question still remains: Is the morphological improvement in this case of physiological significance?

As early as 1884 D'Arsonval (cited by Harris and Benedict, 1919, p. 136) stated that the physiological surface of the animal was not the same as the "physical." The ventral part of the skin of an animal living outdoors which radiates to the ground may have a heat loss very different from the dorsal part radiating to the sky. A similar view has been expressed by Carman and Mitchell (1926, p. 380). In order to be exact, the different rate of radiation resulting from different colors of the covering should be considered. Begusch and Wagner (1926) indeed claim that the heat output of dark-colored guinea pigs is 124 per cent of that of light-colored guinea pigs; and recently Deyghton (1929, p. 151) put forward a similar idea, mentioning that, according to de Almeida, negroes in Brazil had a metabolism about 8 per cent higher than that of white men. These statements, especially in their relation to the color of the skin, may not be above criticism (see Du Bois, 1930, p. 222), but certainly Benedict and Talbot (1921, p. 160) are correct in writing that: "The physical and physiological factors influencing the heat loss from the surface of the human body are so different at different parts of the body as to preclude any generalization that equal areas result in equal heat loss."

It might be thought that on the average the "physiological surface" would be a constant part of the geometrical surface; and for an approximation this supposition is probably correct; but there does not seem to be enough reason for the belief that this proportionality is so accurate as to justify improvements in methods or formulas which allow the

[11] Mitchell (1929, p. 440) found the area of the skinned carcass of the rat to be 430 sq. cm. The unstretched skin measured 536 sq. cm. A moderate stretching increased the area to 630 sq. cm.

determination of a "true" geometric surface area with a few per cent less variation than has been possible hitherto.

If a cat is curled up for sleep, as it is during a considerable part of its life, the calculation of its surface as a sphere is, from the point of view of heat loss, probably better than the improved calculation according to Meeh, because in the latter case one calculates the ventral part of the skin as surface, although in the curled position this is certainly not a cooling surface comparable to the dorsal part.

Thus, even if the surface of the skin were well defined, the improvements in measuring it may not be significant for the question of body size and metabolism.

The development of as many different formulas for calculating the surface as there are species concerned, or even more, physiologically not only is a doubtful improvement but has a definite *disadvantage*. The present situation in reducing the metabolism to the unit of body surface is similar to the general condition of measuring lengths in the Middle Ages when the size of the foot varied from country to country and in referring to a certain length, one therefore had to be sure which foot was used. This situation is present in measuring the metabolism even within one species. If it is stated, for example, that a steer has a metabolism of m calories per square meter of body surface, it is necessary to find out whether that surface area has been calculated on the basis of Meeh's formula and, if so, which constant has been used. The calculation may have been made according to Moulton, or according to Hogan's formula; it is also possible that the author has a formula of his own, or that he determined the surface of his steers directly. And if the method of determining the surface is known, further difficulty arises when one attempts to compare this result with others also obtained on steers, but on the basis of different methods for the surface determination.

One may readily come to the conclusion that improvements in determination of surface lead to a labyrinth, and that it might be better to go back and relate the metabolism to the unit of body weight, giving up the comparison of the metabolism of animals so different in size that the reduction to the unit of weight might imply a considerable error. This has recently been done by Benedict and Riddle (1929) in their work on the metabolic rate of pigeons. But this step out of the chaos should be the start rather than the end. Benedict and Riddle also use a common unit, the weight; they can do so as long as their individuals are similar in size. But they cannot, for example, directly compare the metabolism of ring doves and pigeons. And if within one species they had material with large variations in body size, the question would also arise whether it is correct to calculate on the basis of the

proportionality of metabolism to weight. In a good deal of metabolism work this question cannot be avoided. The comparison of the metabolism of different animals cannot be given up, and therefore the search for a common basis for comparing the metabolism of animals different in size cannot be given up; for on this basis alone can studies be made of other influences on the metabolism, such as age, sex, and condition of body.

Krogh (1916, p. 140) has proposed to reduce the metabolism to the unit of W^n instead of the body surface. Stoeltzner (1928) uses the same unit when he calculates for medical purposes the energy requirement of man as $160 \times W^{2/3}$. Brody, Comfort, and Mathews (1928, p. 23) also prefer the use of a power function of the weight as a unit for calculating the metabolism. The last-mentioned authors write: "We do not quite see the logic involved first in relating area to body weight, then computing area from body weight, and finally relating heat production to the computed area. Why not relate heat production to the body weight directly?" Mitchell's objection (1930*a*, p. 444) to this proposal is that it ignores the physical significance of the relation between surface and heat production. Indeed, the empirical result that the metabolism is proportional to a power function of the weight is independent of any theory about the physical background of this relation.

But the use of W^n as the unit of body size for metabolism does not necessarily exclude a physical significance of the relation between surface and heat production. If the surface is calculated according to Richet as $4.84 \times W^{2/3}$ and if the heat loss is proportional to the surface, it is, as a matter of course, also proportional to $W^{2/3}$. A real difference in opinion can occur only if the surface of different animals cannot be expressed as the same power function of the weight.

The surface per unit of $W^{2/3}$, or the Meeh constant $\left(k = \frac{S}{W^{2/3}}\right)$ is a measure for a relatively large or small surface of animals; this term, which is about 10 for most animals, goes up as high as 13 for the rabbit, showing the influence of its large ears. Calculating the metabolism simply to the ⅔ power of the body weight, an abnormally high value for the metabolism of rabbits would be expected. This is not the case. Voit (1901, p. 116) found a basal metabolism for the rabbit of only 776 Calories per square meter using the Meeh formula $S = 12.9\ W^{2/3}$. It is to be stated, however, that the value of 776 is still too high. Voit writes that this value would have been much lowered had he averaged all data available on the basal metabolism of rabbits. If the area of the ears is subtracted from the body surface, the metabolism of the

rabbit fits better into Rubner's scheme of 1,000 Calories per square meter, for it is then 917 Calories (Lusk, 1928, p. 124). In determining the surface of the rabbit, it is therefore doubtful whether or not the area of the ears belongs to that surface. This means a difference of 20 per cent, and it may be asked: What do we gain if we can develop a method which allows us to determine the surface area to within few per cent accuracy, if an amount of 20 per cent is in any way doubtful? A physiological reason may be found for subtracting the area of the rabbit ears from its total surface area, but what remains of the surface law if corrections of this kind have to be made? What remains is in accordance with the empirical result of table 1: A general influence of body size on the metabolism which may be related to W^n as well as, or even better than, to the actual surface.

It may therefore be concluded: Although no definite power function of the body weight can as yet be given as the best unit to which the metabolism of animals which differ in size may be calculated, there is reason to give up the unit of body surface because it is not well defined and because its strict application tends to obscure rather than to clear up the knowledge of the influence of body size on metabolism. Any unit of body weight from the ⅔ up to the ¾ power is preferable to the unit of body surface because a power function of the body weight is so much better defined than the unit of body surface and because its general application to all homoiotherms opens such a wide field from the point of view of comparative physiology that even considerably greater deviations from the mean by the use of W^n instead of the surface, would be outweighed.

The Intraspecific Application of the Interspecific Results.—The best-fitting unit of body size for comparing the metabolism of rat, man, and steer has been found to be $W^{3/4}$. Is there objection to using this unit for comparisons within one species?

From a table on the metabolism of dogs given by Rubner (1928, p. 164) it follows that the metabolism per square meter of body surface is on the average somewhat higher in the smaller dogs than in the larger ones. The coefficient of tendency, the term τ (see p. 320), is in this case -0.362 per cent of the mean.

From another table by Kunde and Steinhaus (1926, p. 128) giving also results obtained on dogs by Rubner the contrary conclusion would be drawn, namely a larger metabolism per square meter of body surface in the larger dogs, the term τ being $+0.200$. As Rubner calculated the surface on the basis of Meeh's formula, the result is applicable also for the ⅔ power of the weight.

Figures given by Richet (1889, p. 222) for the metabolism of rabbits show that the metabolism per unit of $W^{2/3}$ is decreased with increasing body weight. These data, as well as the first-mentioned table of Rubner, though confirming the general influence of body size on metabolism and the theory that this influence is more closely related to the ⅔ power of body weight than to body weight directly, seem to be in contradiction to the more special interspecific result, that the best-fitting unit of body weight is from $W^{0.72}$ to $W^{0.74}$ or approximately the ¾ power.

As age and body condition (especially fat content) were not taken into consideration, their data do not indicate whether or not the heavier animals were on the average also the fatter and older ones. Hence no conclusive answer to the question with regard to rabbits or dogs can be obtained though these two species would be especially suitable for intraspecific studies on the relation of body size and metabolism.

The data on the 136 men in the biometric study of Harris and Benedict (1919, p. 40, ff.) have been arranged in eight groups according to body weight. The age was well equalized among these groups. The same has been carried out for the 103 women. In this case the group of the heaviest women has been omitted from calculation because the average age of this group was much higher than the average age of the other groups. The average metabolism and weight of those groups have been submitted to the same calculation as the data on the thirteen groups in table 1. The result of this calculation is shown in table 7.

TABLE 7

Basal Metabolism of Human Beings
Calculated to Different Units of Body Size

Unit of body size	Average basal metabolism Cals. per 24 hours per unit of body size		Coefficient of tendency in per cent of mean (τ)	
	Men	Women	Men	Women
W (Body weight)	25.7	25.3	−0.537	−0.778
$W^{3/4}$	72.5	67.8	−0.188	−0.339
$W^{0.7}$	80.1	82.7	−0.108	−0.242
$W^{0.6}$	134.9	122.9	+0.053	−0.056
$W^{0.5}$	205.5	182.7	+0.302	+0.130
$S = 12.31 \times W^{2/3}$ (Meeh)	830	767	−0.040	−0.177
$S = W^{0.425} L^{0.725}$ (DuBois)	925	857	+0.158	+0.125

The two main results obtained by interspecific comparison seem to be confirmed within the human species: (1) the metabolism is more closely related to the surface or to the ⅔ power of the weight than to its first power; (2) there is no evidence that the surface of the skin is a better unit for the calculation of the metabolism than some power function of the weight would be.

The best-fitting unit for calculating the metabolism of human beings seems to be a power function close to $W^{0.6}$. This is not in accordance with the result obtained by interspecific comparison where the term $W^{0.72}$, or even $W^{0.74}$, if ruminants are included, was found to be the best fitted.

As already mentioned, the results in table 7 within the human species may be obscured by the influence of other factors. I have attempted to eliminate two of those factors by calculation, namely *age* and *build*, the two influences which are considered besides weight in the regression equation of Harris and Benedict for the prediction of human metabolism.

The calculation has been carried out as follows:

Influence of Age in Man.—The influence of age on the metabolism has been calculated from the material which Benedict (1915, p. 284) has selected for this purpose. Three results have been omitted in order to get rid of the possible influence of stature. The calculation is shown in table 8.

TABLE 8

AGE AND METABOLISM IN MAN

Group	Age		Weight, kg.	Height, cm.	Specific stature*	Total Cals. per 24 hours
	Range	Average years				
Average of 14 men	16–41	26.0	60.3			1,578
7 younger men	16–24	20.3	60.9	168	42.9	1,631
7 older men	26–41	31.7	59.7	168	43.1	1,525
Difference		11.4	−1.2	0	0.2	106
Difference due to weight†						23
Difference due to age						83

Difference due to age per year $= \frac{83}{11.4} = 7.3$ Cals.

Per cent of average metabolism (coefficient of age) $\frac{7.3}{1{,}578} \times 100 = 0.46$ per cent.

* For definition see p. 343.

† The correction for the difference in weight has been calculated on the basis of the equation $\frac{dM}{dW} = 0.73\frac{M}{W}$ (see p. 320) which was derived from table 1.

From a graph given by Harris and Benedict (1919, p. 120) it may be concluded that the heat production per square meter of body surface decreases in men 0.37 per cent of the average (926 Cals.) for each year increase in age; the corresponding figure for women is 0.34 per cent.

The advantage of obtaining the coefficient of age on 14 men as described above is that other influences are well excluded. The advantage of the last-mentioned figures is that they are obtained from a larger number of individuals.

Considering the variations which are to be expected, the second place of the figure may be omitted, the decrease of metabolism per yearly increase in age assumed to be 0.4 per cent of the metabolism at the age of 30.

The metabolism differs according to whether a person is stout or slim, as suggested by Benedict. Stature is no adequate measure for an influence of that kind, for it depends on weight itself; stature must be considered in relation to body weight.

In animals of different size which are similarly built, the quotient of body length (or height in man) and body weight would still depend on weight. The smaller the animals the larger it would be. A good unit, however, which expresses in one figure how stout or slim an individual is, and which is independent of the body size, is the quotient of body length (L) in centimeters divided by the cube root of body weight (W) in kilograms. This term $\frac{L}{W^{1/3}}$ may be called the *specific stature*.[12] As the weight is proportional to the volume, the cube root of it is proportional to a linear dimension, thus the specific stature is a term without dimensions.

In order to determine the influence of build on the metabolism, the results on the 136 men reported by Harris and Benedict (1919) have been arranged according to the specific stature into two groups, as shown in table 9.

TABLE 9

INFLUENCE OF SPECIFIC STATURE ON METABOLISM IN MAN

Group	Specific stature $\frac{L}{W^{1/3}}$	Body weight, (W), kg.	Height (L), cm.	Age, years	Calories produced in 24 hours			
					Total	per $W^{2/3}$	per $W^{0.7}$	per $W^{0.75}$
Average, 136 men	43.4	64.1	173	27.0	1,631	102.0	88.8	72.2
68 slim men	44.8	59.1	175	25.9	1,567	103.3	90.1	73.5
68 stout men	41.9	69.1	172	28.1	1,695	100.7	87.6	70.9
Difference	+2.9	−10.0	+3	−2.2	−128	+2.6	+2.5	+2.6
Difference due to age*					− 14	−0.9	−0.8	−0.6
Difference due to specific stature					−142	+1.7	+1.7	+2.0
Difference per unit of specific stature						+0.59	+0.59	+0.69
Per cent of the average (coefficient of build)						*per cent* +0.58	*per cent* +0.66	*per cent* +0.96

* 2.2x0.4 per cent = 0.88 per cent of the average.

[12] The inverse of the specific stature has been used by Pirquet and adopted by Cowgill and Drabkin (1927, p. 41) as a measure for the state of nutrition.

The coefficient of build, i.e., the per cent variation in metabolism per unit of variation of specific stature, differs according to whether the influence of size is assumed to be related to the ⅔ or to the ¾ power of the weight, because, on the average, the heavier persons are also the stouter and probably fatter ones.

If the average metabolism of the 8 groups of men mentioned on page 341 is reduced to the same age and the same build by means of the coefficient of age of 0.4 per cent and a coefficient of specific stature of 1 per cent, then the logarithmic relation between body weight and metabolism may be calculated as shown in table 10.

TABLE 10

LOGARITHMIC RELATION BETWEEN BODY WEIGHT AND METABOLISM IN MAN

Group	W	Average log W	Cals. corrected	Log of corrected Cals.
Average 136 men	64.1		1,635	
68 light men	56.3	1.74816	1,495	3.17422
68 heavy men	71.9	1.85460	1,775	3.24802
Difference		+0.10544		+0.07380

$$\frac{\Delta\ (\log\ \text{calories})}{\Delta\ (\log W)} = \frac{0.0738}{0.10544} = 0.70$$

From this calculation the best-fitting unit of body size for comparisons of metabolism within the human species appears to be $W^{0.70}$. The analogous calculation by the use of the coefficient of specific stature of 0.58 per cent shows $W^{2/3}$ as the best-fitting unit.

From the result just mentioned the ⅔ power of the weight seems preferable to the ¾ as unit for human metabolism. A conclusive answer on the question which of the two power functions fits better cannot, however, be given on the basis of the available data. Both the ⅔ power of weight with a coefficient of build of 0.6 per cent and the ¾ power of weight with a coefficient of build of 1 per cent may be tested by their accuracy in predicting human metabolism.

For that purpose the metabolism is formulated in the following equation:

$$M = c \times W^n(1+\alpha(A-a)+\varphi(s-S)+\ldots\ldots)$$

where

M = basal metabolism at temperatures above the critical
c = coefficient of species and sex
W = body weight
n = exponent ⅔ or ¾
α = coefficient of age
A = standard age (arbitrarily chosen constant)
a = actual age
φ = coefficient of build
S = standard specific stature (arbitrarily chosen constant)
s = actual specific stature

This equation expresses three assumptions:

(1) That the metabolism of a person of standard age and specific stature has a metabolism proportional to the nth power of its body weight.

(2) That for each year above or below the standard age, the metabolism is decreased or increased by the same part α of the metabolism at standard age and build.

(3) That for each unit of specific stature above or below the standard specific stature, the metabolism increases or decreases by the same part φ of the metabolism at standard age and build.

It may be found in later investigations that other influences can be measured and added to the equation—for example, the relative fat content of the body, which is now considered only insofar as it influences the specific stature.

The factor c has been obtained as follows:

The average weight of the 136 men in the study of Harris and Benedict (1919, p. 57) was 64.1 kg; the ¾ power of this average is 22.65. The total heat production per day was on the average (Harris and Benedict, 1919, p. 67) 1,631.7 Cals.; thus the average heat production per unit of the ¾ power of the average weight was 72.04 Cals. This is for an average age of 27 years. For a standard age of 30 years the metabolism would be lower—namely, according to the coefficient of age previously developed, $\frac{72.04}{1+0.004\times 3} = 71.2$. This is the factor c for the calculation on the basis of $W^{3/4}$. The corresponding factor for $W^{2/3}$, calculated similarly, is 100.7. The standard build has been calculated by dividing the average height by the ⅓ power of the weight. The prediction equation for the metabolism of man is thus obtained:

(1) $M = 71.2\times W^{3/4}[1+0.004(30-a)+0.01\ (s-43.4)]$
(2) $M = 100.7\times W^{2/3}[1+0.004(30-a)+0.006\ (s-43.4)]$

The analogous calculation has been applied to the data on the 103 women in the study of Harris and Benedict. The prediction in this case may be made according to the equations:

$$M = 65.8 \times W^{3/4}[1+0.004(30-a)+0.018\ (s-42.1)]$$
$$M = 92.1 \times W^{2/3}[1+0.004(30-a)+0.014\ (s-42.1)]$$

The daily heat production predicted according to the four equations was compared with the corresponding data actually observed. In order to show the influence of correction for age and specific stature on the accuracy of prediction, the uncorrected heat production on the basis of the power function of the weight was also compared with the actual heat production.

The average deviation between predicted and observed heat production, irrespective of the sign in per cent of the observed heat production, is given in table 11 together with the square root of the mean square deviation of the observed from the predicted. The corresponding data resulting from the prediction of the metabolism by the regression equations of Harris and Benedict are added for comparison.

TABLE 11

ACCURACY OF PREDICTION OF HUMAN METABOLISM

Basis of calculation	Sex	Formula	Average deviation $\frac{\Sigma d}{n}$	$\sqrt{\frac{\Sigma d^2}{n}}$
$W^{3/4}$ corrected for age and build	Men	$M = 71.2 \times W^{3/4}\ [1+0.004(30-a)+0.01(s-43.4)]$	4.90	6.16
$W^{2/3}$ corrected for age and build	Men	$M = 100.7 \times W^{2/3}\ [1+0.004(30-a)+0.006(s-43.4)]$	5.00	6.17
Harris and Benedict 1919	Men	$M = 66.4730+13.7516\ W+5.0033L-6.7750a$	4.98	6.23
$W^{3/4}$ uncorrected	Men	$M = 71.2 \times W^{3/4}$	6.16	7.72
$W^{2/3}$ uncorrected	Men	$M = 100.7 \times W^{2/3}$	6.01	7.55
$W^{3/4}$ corrected for age and build	Women	$M = 65.8 \times W^{3/4}\ [1+0.004(30-a)+0.018(s-42.1)]$	6.42	7.94
$W^{2/3}$ corrected for age and build	Women	$M = 92.1 \times W^{2/3}\ [1+0.004(30-a)+0.014(s-42.1)]$	6.37	7.84
Harris and Benedict 1919	Women	$M = 655.0955+9.5634\ W+1.8496L-4.6756a$	6.27	7.88
$W^{3/4}$ uncorrected	Women	$M = 65.8 \times W^{3/4}$	9.31	11.80
$W^{2/3}$ uncorrected	Women	$M = 92.1 \times W^{2/3}$	8.53	11.42

There could hardly be a better recommendation for either one of the four equations developed herein than the fact that they predict the metabolism with practically the same degree of accuracy as the empirical regression equations of Harris and Benedict (1919, p. 227).

The criticism of Krogh, presented by Boothby and Sandiford (1924, p. 80) that the terms of Harris and Benedict are of purely statistical nature does not apply to the equations developed in this paper; the coefficients in the latter equations have a certain physiological meaning.

Reducing the equation for the women to the average specific stature of men, the two results can be compared directly:

$$\text{for women} \quad M = 67.4 \times W^{3/4}[1+0.004(30-a)+0.018\ (s-43.4)]$$
$$\text{for men} \quad M = 71.2 \times W^{3/4}[1+0.004(30-a)+0.010\ (s-43.4)]$$

where

W = weight in kg

a = age in years

$$s = \text{specific stature} = \frac{\text{stature in cm}}{\text{weight}^{1/3}}$$

On the basis of the same specific stature the ratio of the metabolism of men and women would therefore be as 71.2 : 67.4 = 1 : 0.95. Without reduction to the same specific stature the ratio is wider—namely, 71.2 : 65.8 = 1 : 0.93, because on the average the women have a lower specific stature.

If the metabolism of the 136 men and 103 women studied in the Carnegie Nutrition Laboratory is reduced to a standard age and standard specific stature, any power of the body weight from the ⅔ to the ¾ serves as well as or better than the unit of body surface for expressing the influence of body size on metabolism.

Therefore there is reason to apply for intraspecific calculation the same power of the weight (within the mentioned limits) which may by interspecific comparison be found the best.

GENERAL CONCLUSIONS

The result of recent work on the basal metabolism of different species and the critical review of the fundamentals of the surface law leads to the suggestion that the surface law should be replaced by a weight-power law. A power function of the body weight gives a better-defined unit for measurement than the unit of body surface.

From comparison within the human species it follows that the metabolism may be formulated thus:

$$M = C \times W^{n}[1+\alpha(A-a)+\varphi(s-S)+\ldots\ldots\ldots]$$

Not only is it probable that the metabolism of all homoiotherms may be expressed in the same scheme but it seems that the same exponent of the bodyweight (n) may be used for interspecific comparisons as well as for comparisons within one species.

Research on metabolism would be much more economical, i.e., less time-consuming, if the term W^n could be settled so that all authors would express their results on the same basis. This task would require further systematic experimental work, especially with regard to the critical temperature. It would call for international cooperation and agreement.

SUMMARY

A table with the results of recent work on metabolism of different animals from the ring dove and the rat to the steer shows a closer relation of the basal metabolism to the $\frac{3}{4}$ power of body wieght than to the geometric surface of the animal.

In order to study the question whether or not there is a theoretical reason for maintaining the surface of the skin as the basis for comparing the metabolism of animals which differ in size, four theories of the surface law, namely, temperature regulation, nutritive surface, composition of the body, and rate of blood circulation, are discussed.

It is demonstrated that the animal can vary its specific insulation to a considerable degree, and that therefore an accurate relation between surface and heat flow, according to Fourier's Law, is not to be expected.

However, as the possibilities of altering the specific insulation are practically limited, the heat-loss theory for cold climates and the overheating theory for hot climates stand criticism for *approximate* comparison of the heat-production of animals which differ sufficiently in size.

Basing the surface law on the nutritive surfaces, the cell surfaces, or the protoplasm structures has been shown to be without warrant.

Differences in the composition of the body, inert fat, active protoplasm, and amount of blood, though unquestionably affecting metabolism, cannot explain the considerable influence of body size on the metabolism of different kinds of animals. The fact that the basal metabolism of warm-blooded animals is approximately proportional to the $\frac{2}{3}$ or the $\frac{3}{4}$ power of the body weight is a matter governed by the organism as a whole; it cannot be derived from a summation of the vital functions of the cells or other parts of the body.

On the basis of the similarity in the building plan of all warm-blooded animals and of the limited velocity of muscular contraction, it may be conceived that the intensity of blood flow, and hence the intensity of oxygen transport to the tissues, is related more closely to a lower power of body weight than unity.

The biological explanation of the relation of body size and metabolism may be expressed as follows: In natural selection those animals are the fittest in which the caloric requirements are in harmony with the

hemodynamic possibilities of oxygen transport. This harmony seems to be established when the logarithm of the metabolism is proportional to the logarithm of body weight.

No theoretical evidence has been found to indicate that the metabolism of animals should be related exactly to the surface area of their skin.

For the sake of precision, the metabolism of animals should not be given in terms of body surface, because this term is not well defined.

A simple equation probably applicable to all homoiotherms and characterizing the metabolism by three coefficients (sex and species, age, specific stature) gives a prediction of the metabolism of man on the basis of the $\frac{2}{3}$ or the $\frac{3}{4}$ power of body weight with practically the same degree of accuracy as by the empirical regression equation of Harris and Benedict. This result strengthens the hypothesis that the intraspecific relation of body size and metabolism follows the same logarithmic rule as has been found by interspecific comparison.

It is suggested that the heat production of all warm-blooded animals should be expressed in terms of the same power of the body weight and that for the sake of economy in research the question of the best-fitting exponent ($\frac{2}{3}$ to $\frac{3}{4}$) should be studied in order to find a unit for measurement which might be adopted internationally.

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METHODS FOR THE ISOLATION OF BRUCELLA ABORTUS[1]

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CONTENTS

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INTRODUCTION

Owing to the greatly increased interest in *Brucella abortus* since it has been definitely established as a human pathogen and since its economic importance has been more widely recognized, the attempted isolation of the organism from suspected materials has become a routine practice in a large number of laboratories not previously interested in the *Brucella* group.

The procedures given below, together with some experimental data, are based on several years' work of this and other laboratories interested in bovine–abortion control. For the most part the methods are those in general use or modifications of such. These methods have uniformly given satisfactory results in research and also in routine diagnosis such as certified-milk inspection and control work in other dairy herds. Because of these results, which have been obtained from the inoculation of several thousand guinea pigs, it was thought that a detailed description of the technique might be of value, in whole or in part, to other workers.

In addition to outlining the methods used, an attempt has been made to compare and evaluate the various procedures.

DIRECT CULTURE METHOD

Although the isolation of *Brucella abortus* by guinea-pig inoculation, especially from milk, has been the most widely accepted method for many years, from time to time the use of culture media for the direct isolation of the organism from suspected material has been advocated. The most recent work on this method is that of Huddleson, Hasley, and Torrey.[13] For the isolation of *Br. abortus* from milk, these workers use liver-infusion agar to which is added a saturated aqueous solution of gentian violet in the proportion of 1 part to 10,000 parts of culture medium. The medium is allowed to harden in petri dishes, and 0.1 cc of cream is spread over the surface of the plate. The dye inhibits the growth of most of the other bacteria in the milk, but allows *Br. abortus* to develop. The advantages of this method of isolation are numerous. The saving of time in reaching a final diagnosis, the low cost of plates as compared with the cost of guinea pigs, and the cost of their care and of the equipment necessary for maintaining them over a long period of time, as well as the time consumed in post-

mortem examinations, are all arguments in favor of the direct isolation by means of culture media. On the other hand, there has been a general feeling on the part of many laboratory workers that guinea-pig inoculation is the more delicate test for the detection of *Br. abortus,* especially in material containing other organisms.

Because of the lack of sufficient definite data on the relative merits of the two methods, it was decided to make cultures of milk from a large number of samples which were being used for guinea-pig inoculations.

The medium selected for this work was 'chocolate' or cooked-blood agar. This preparation, probably first described by Fleming(9) for the isolation of influenza bacilli, has been used in this laboratory since 1919, and has given excellent results in the primary isolation of *Brucella abortus* and many other organisms. Comparisons have been made from time to time between the cooked-blood agar and other media, particularly liver-infusion agar, but in all cases the cooked-blood agar has yielded the most uniform results.

Preparation of Culture Medium.—The cooked-blood agar is relatively simple to prepare, and it has a very definite advantage in that the constituents may be stored for a considerable length of time and fresh medium made in small quantities in a very short time. The variation between lots of this medium is relatively slight compared with infusion media. The method of preparation follows.

Two per cent of agar is added to ordinary nutrient broth, adjusted to a pH of 7.4, and sterilized. When the agar has cooled to below 70° C, 8 per cent by volume of sterile defibrinated horse blood is added. This mixture is placed in a water bath at 70° C for 10 minutes, after which it is transferred to a sterile covered funnel, to the end of which a rubber tube and pinch-cock has been attached. The medium is immediately distributed to sterile tubes and petri dishes.

Although the final pH will usually be about 6.8, a check may easily be made just previous to tubing the medium, by centrifuging a tube of the medium in a trunnion containing warm water, and testing the clear supernatant agar with an indicator.

For the isolation of *Brucella abortus* from material such as milk, which contains other organisms, 0.1 cc of a saturated alcoholic solution of gentian violet is added to every liter of medium, making a final dilution of the dye approximately 1 to 208,000.

Comparison of Culture and Guinea-Pig Injection.—The milk used for the comparative tests was taken from 22 cows, most of which

were positive to the agglutination test for *Brucella abortus*. The milk from each quarter of the udder was treated as a separate sample, and the results below represent from one to four sets of samples from each cow. The sample was centrifuged as described in the paragraph on preparation of material for injection, and the cream and sediment thoroughly mixed. The tubes containing the inoculum were kept iced except for a few minutes just previous to planting or injecting. At this time the material to be used was placed in a water bath at or slightly below 38° C, so that a representative sample could be obtained.

One-tenth cc of the material was placed on the surface of a gentian-violet cooked-blood-agar plate and spread over the surface by means of a wire dally. The plates were incubated in air overnight, then placed in 10 per cent carbon dioxide and incubated for four more days. At this time the *Brucella abortus* colonies were counted, and a typical colony from each positive plate transplanted, later being tested with known positive and negative *Br. abortus* antiserum. Suspicious colonies from plates having no typical *Br. abortus* growth were transplanted, and if the organisms resembled *Br. abortus* when stained, were also tested by agglutination.

The guinea pigs were inoculated intraperitoneally with 2 cc of the material, and slaughtered at the end of 6 weeks. The procedure outlined below for the post-mortem examination and isolation of *Brucella abortus* was followed.

A total of 247 parallel tests were made with plates and guinea-pig inoculation. Of these, 100 proved positive for *Brucella abortus* by one or both methods.

One hundred guinea pigs proved to be infected, and *Brucella abortus* was recovered from the spleen in each case. Only 71 of the plates yielded *Br. abortus* and in no case did the guinea pig prove negative where the plate inoculated with the same material was positive. However, in another series of 36 samples in which the guinea pigs were injected approximately 24 hours after the plates were made, *Br. abortus* was obtained by the plate method in 5 samples which were negative by guinea-pig injection. Five other samples were proved positive by guinea-pig inoculation, but failed to show *Br. abortus* colonies on the plates. The number of positive samples by each method was 17 and the negative 19.

As considerably more time and care was taken, both in the preparation of the medium used and in the examination of suspicious colonies, than is feasible for ordinary routine examination, it seems probable that failure in at least 25–30 per cent of the cases must be expected when the culture method alone is depended upon.

In addition to the failure of the culture medium to detect *Brucella abortus* in some materials which may be proved to be positive by guinea-pig inoculation, the occasional occurrence of dye-tolerant contaminants in large numbers in some suspected materials may be a source of error, or of considerable annoyance. When contamination by organisms with colonial appearance resembling that of *Br. abortus* occurs, the time and labor involved in ascertaining the identity of the organisms soon approaches that necessary for guinea-pig inoculation.

The ever present danger that a given lot of medium may be unsatisfactory for the growth of *Brucella abortus,* because of unsuitable H^+ concentration, poor physical characteristics, etc., also adds to the disadvantages of this method. This can only be avoided by testing each lot with cultures of *Br. abortus*, which takes several days and allows the medium to become somewhat dry.

While the culture method, in our opinion, cannot replace animal inoculation, it would seem to have distinct value as a supplement to the slower method. By the use of both methods, those animals inoculated with materials which prove to be definitely positive by the culture method may be destroyed without examination, and the final decision on materials which give negative or unsatisfactory results on the plates may be reserved until the guinea pigs are autopsied. Or, in order to avoid the injection of a large number of guinea pigs, milk may be preserved with 1 per cent boric acid or held at ice-box temperature until the results of the cultures have been obtained. Those samples which prove negative may then be injected into guinea pigs.

GUINEA-PIG INJECTIONS

Selection of Animals.—In this laboratory male guinea pigs weighing from 350 to 400 grams have been found to be the most satisfactory test animals. An animal of less than 300 grams is apt to show ill effects from the large quantities of inoculum, and from the handling necessary at the time of inoculation. Male pigs are preferred because of the characteristic *Brucella abortus* lesions produced in the testicles, and because they seem somewhat more suspectible to infection with small numbers of organisms. Table 1, although it includes only a small number of tests, indicates the relative susceptibility of male and female guinea pigs when inoculated with naturally infected milk.

The number of organisms per gram of spleen, as given in table 1, was estimated by the method described by Hagan.[12] This consists in grinding a weighed portion of the infected spleen and making

dilutions from which plates are seeded. The simple but ingenious apparatus which Hagan described for the grinding of the spleen is made by selecting two common test tubes, one sufficiently smaller than the other to allow it to be inserted within the first. The pestle tube should fit into the outer one rather loosely to avoid having the emulsion creep up between the tubes. The tubes may be fitted together, weighed, capped with paper, and sterilized. When ready for use, a portion of the spleen is placed in the larger tube, and after replacing the inner tube, the weight of the spleen is determined. After grinding,

TABLE 1

COMPARISON OF POST-INOCULATION PERIODS AND THE RELATIVE SUSCEPTIBILITY OF MALE AND FEMALE GUINEA PIGS TO BRUCELLA ABORTUS IN MILK

Milk from Cow No.	Killed at	Sex	Weight of spleen in grams	*Br. abortus* per gram spleen	Agglutinin titre (1-25, 1-50, etc.)	Lesions
A652	4 weeks	♂	1 555	6,850	+±--------	−
		♀	1.237	....	----------	−
	6 weeks	♂	4 175	578,400	+++++-----	+
		♀	1.300	....	----------	+
	8 weeks	♂	3 035	147,800	+++++++++-	+
		♀	1.210	446,300	+++-------	Slight
A656	4 weeks	♂	0.802	15,000	+++±∓-----	+
		♀	0.945	....	∓---------	Slight
	6 weeks	♂	0.845	....	∓---------	−
		♀	1.095	....	----------	−
	8 weeks	♂	0.645	307,700	+++++±----	Very slight
		♀	1.025	204,700	+++∓------	Very slight
A659	4 weeks	♂	0.595	9,950	+++-------	−
		♀	1.100	8,100	+++±------	+
	6 weeks	♂	1.020	68,600	+++±------	+
		♀	1.285	700	++±-------	Slight
	8 weeks	♂	5.440	134,700	++++++++++	+
		♀	3.005	40,800	++++++++±-	+

the spleen may be diluted with sterile saline. A convenient initial dilution is 10 cc per gram of spleen.

Testing of Guinea Pigs.—For several years it was the practice in this laboratory to bleed all guinea pigs a few days previous to inoculation, and to test the blood for *Brucella abortus* agglutinins. This was discontinued, but later two animals, inoculated with materials unlikely to have contained *Br. abortus* and kept in the laboratory for several months, became infected with *Br. abortus*. Since that time all guinea pigs used for *Br. abortus* experiments have been tested before inoculation. All animals mentioned in this paper were so tested. However, the results of several thousand tests are now on record, and in no case has an animal shown even a trace of agglutinin content

in its blood. Because of these continuously negative results it seems unlikely that guinea pigs brought into the laboratory within a few weeks previous to inoculation will be found to be infected with *Br. abortus.* Animals which have been raised or held for a considerable length of time in a laboratory where *Br. abortus* infected animals are kept should be tested before being used.

Blood may be drawn from the heart for testing, but animals treated in this manner should be kept several days, subsequent to the bleeding, before inoculation. The following method, a modification of that described by Seddon,[17] has the advantage of not affecting the animal to any appreciable extent.

Ordinary agglutination test tubes are marked by means of a file at points indicating 4.5 cc and 5 cc. The tube is filled to the 4.5 cc mark with 0.85 per cent salt solution containing 1.00 per cent sodium citrate and 0.50 per cent phenol. An ear vein of the test animal is cut and the edge of the tube is rubbed repeatedly upward over the cut to collect the slowly oozing blood until the mixtures reaches the 5 cc mark. The guinea pig is then ear-tagged and the tag number placed on the cork of the test tube.

After standing a short time, the corpuscles settle sufficiently so that the clear liquid, representing a serum dilution of approximately 1–20, may be drawn off, and the agglutination test performed. Blood may also be collected from the ear and allowed to clot in a capillary tube. Sufficient serum may be obtained in this manner to perform the agglutination test.

Housing to Prevent Accidental Infection.—During the course of an experiment in which large numbers of guinea pigs were inoculated with materials containing *Brucella abortus,* circumstantial evidence seemed to indicate that, in a very small percentage of cases, guinea pigs inoculated with materials free from *Br. abortus* became infected by contact with diseased pigs. In light of Surface's[20] report of a spontaneous outbreak of *Br. abortus* infection among guinea pigs, it was decided to keep each inoculated animal in a separate cage. For this purpose ordinary cracker tins 10½ by 10½ by 8 inches, with hinged tops, have been found very satisfactory after the top has been perforated with ½-inch holes.

Although the possibility of infection by contact is present, it apparently does not occur readily. Hagan[12] was unable to infect animals by keeping them in close contact with animals of the same sex. He did, however, in one case succeed in producing infection in a female guinea pig which was kept with an infected male. In table 2 the results of a similar test, in which we also were unsuccessful in the attempt to transmit the disease by contact, are given.

TABLE 2

RESULTS OF POST-MORTEM EXAMINATIONS AND CULTURES FROM GUINEA PIGS IN CONTACT FOR 6 WEEKS WITH GUINEA PIGS INFECTED WITH BRUCELLA ABORTUS

Cage	Treatment	Guinea pig No.	Sex	Blood serum*	Lesions	Spleen culture	Urine culture
A	Inoculated†	7222	♂	Died; no test	+	+	+
		7223	♂	++++	+	+	+
		7224	♂	++++	+	+	+
	Not inoculated						
	Ear tagged	7225	♂	----	–	–	
		7226	♂	----	–	–	
	Ear tagged; skin of abdomen abraded weekly	7227	♂	----	–	–	
		7228	♂	----	–	–	
	No ear tags; no visible abrasions	7256	♀	----	–	–	
		7257	♀	----	–	–	
B	Inoculated†	7229	♀	++++	+	+	+
		7230	♀	++++	±	+	+
		7231	♀	++++	+	+	–
	Not inoculated						
	Ear tagged	7232	♀	----	–	–	
		7233	♀	----	–	–	
	Ear tagged; skin of abdomen abraded weekly	7234	♀	----	–	–	
		7235	♀	----	–	–	
	No ear tags; no visible abrasions	7254	♀	----	–	–	
		7255	♀	----	–	–	
C	Inoculated†	7258	♀	Died	+	+	+
		7259	♀	Died			
		7260	♀	++++	+	+	
		7261	♀	++++	+	+	+
	Not inoculated						
	Ear tagged	7262	♀	----	–	–	
		7263	♀	----	–	–	
	Ear tagged; abdomen shaved and abraded weekly	7264	♀	----	–	–	
		7265	♀	----	–	–	
	No ear tags; no visible abrasions	7266	♀	----	–	–	
		7267	♀	----	–	–	
D	Inoculated†	7268	♂	++++	+	+	+
		7269	♂	++++	+	+	+
		7270	♂	Died	+	+	+
	Not inoculated						
	Ear tagged	7271	♂	----	–	–	
		7272	♂	----	–	–	
	Ear tagged; abdomen shaved and abraded weekly	7273	♂	----	–	–	
		7274	♂	----	–	–	
	No ear tags; no visible abrasions	7275	♂	Died	–	–	
		7276	♂	----	–	–	

* The titre limit of these serums was not determined.

† Inoculated intraperitoneally with 0.5 cc of a suspension of *Br. abortus* No. 80.

PREPARATION OF MATERIAL FOR GUINEA-PIG INJECTION

Milk.—The isolation of *Brucella abortus* from the milk of cows by guinea-pig inoculation was first reported by Schroeder and Cotton[16] and Smith and Fabyan[18] in 1911, and has since been used extensively for the detection of shedder cows in infected herds. It is of importance also in cases of human infection attributed to infected milk; for a positive agglutination test of the blood serum of the cow supplying the milk does not necessarily indicate the presence of the organism in the milk.

TABLE 3

COMPARISON OF THE NUMBER OF BRUCELLA ABORTUS ORGANISMS FOUND IN CREAM AND SEDIMENT OBTAINED BY CENTRIFUGING AND BY GRAVITY

Sample No.	Centrifuged cream and sediment: organisms per cc	Cream and sediment obtained by standing: organisms per cc
1	10,960	2,650
2	1,960	1,060
3	3,750	480
4	140	0
5	3,090	70
6	2,290	510
7	240	100
8	900	1,720
9	1,100	370
10	1,370	130
11	7,080	350
12	170	0
13	520	10
14	440	0

Evans,[7] Carpenter,[5] and others have shown that the number of *Brucella abortus* organisms found in milk is relatively small. For this reason, the direct inoculation of the milk may fail to produce disease in the guinea pigs. Concentration of the bacteria by centrifuging may yield positive results in cases where inoculation of the whole milk would fail to reveal the organism. The method outlined below has been in use in this laboratory for several years, and previous to that time was used in the United States Department of Agriculture Bureau of Animal Industry laboratories in Washington. It has also been outlined by Carpenter. The milk is collected into a sterile, wide-mouthed bottle with aseptic precautions, iced, brought to the labora-

tory, and from 70 to 90 cc of it is transferred to 100 cc sterile centrifuge tubes. After the milk has been centrifuged at high speed for 20 minutes, most of the skimmed milk is discarded by decanting, using a sterile glass rod to prevent the disk of cream from leaving the tube. Two to 4 cc of the skimmed milk, together with sediment and cream, are retained, and the whole thoroughly mixed with the rod. About 3 cc of this concentrate is inoculated intraperitoneally into the guinea pig. A part of the skimmed milk may conveniently be saved, and, after the addition of rennet, be used to determine the agglutinin content of the milk.

If a centrifuge is not available, the milk may be kept overnight on ice, and the cream and sediment mixed in the same manner as in the centrifuged samples. However, comparative tests show that this method is not so efficient as the centrifuge method, as indicated in table 3. This is, however, contrary to the findings of Huddleson, Hasley, and Torrey,[13] who gave the following:

	Cow 8 *Br. abortus* organisms	Cow 84 *Br. abortus* organisms
Centrifuged cream 0.1 cc	207	77
Gravity cream 0.1 cc	552	209

For milk which must be shipped without ice, Gilbert, Coleman, and Groesbeck[10] have proposed the use of 30 per cent glycerine as a preservative. Traum and Henry[21] have found that naturally infected milk kept at room temperature for more than 10 days will still induce *Brucella abortus* infection in guinea pigs when inoculated intraperitoneally, if 1 per cent boric acid is added to the milk at the time it is drawn.

Udder Exudate.—In making a survey of the extent of *Brucella abortus* infection in a dairy herd it is often very desirable to include the dry cows as well as those in active lactation. For several years excellent results have been obtained in this laboratory by the inoculation of the semigelatinous exudate obtained from the udders of dry cows. The information obtained in this manner is especially important in an epidemiological or epizoological study where only a single test of the herd is possible.

Fetal Tissues.—The isolation of *Brucella abortus* from aborted fetuses which are in good condition may usually be accomplished by the use of culture media without recourse to guinea-pig inoculations. However, badly torn or poorly preserved specimens may contain so many contaminating organisms that direct cultures will be badly overgrown. In such cases, small fragments of tissue may be ground in a mortar, suspended in salt solution, and inoculated. If the material

is heavily contaminated, gentian violet in a final dilution of 1 to 200,000 may be added to the suspension and allowed to stand 30 minutes, in order to reduce the number of contaminating organisms and prevent peritonitis in the guinea pig. For ordinary routine examination, the stomach and rectal contents of the fetus are combined and inoculated into one guinea pig, and pieces of the lung and liver are inoculated into another pig.

Vaginal Swabs.—When the placental and fetal materials from an aborting animal are not obtainable, the organism may in some cases be recovered by the inoculation of material obtained by means of a vaginal swab. For such inoculations a convenient swab may be pre-

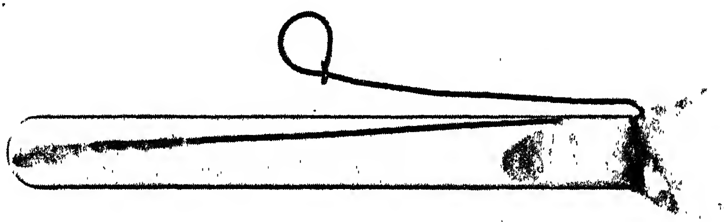

Fig. 1. Sterile swab for obtaining uterine exudate from vagina or uterus of cattle.

pared by wrapping cotton on a stiff wire about 15 inches long. The swab end of the wire is inserted into a large (1 by 8 inch) test tube, the tube being plugged with cotton and the portion of the wire remaining outside the tube bent down against the tube to form a compact package (fig. 1). The whole is wrapped in paper and sterilized. When the charged swab is received in the laboratory, it is removed from the tube and placed in a tube containing 3 or 4 cc of sterile saline solution. After being soaked for 10 to 15 minutes and sufficiently shaken to insure suspension of the material, the salt solution is inoculated into the guinea pig.

Urine.—The sediment obtained by the centrifugalization of urine may be inoculated in the same manner as is milk. In the case of bovine material this procedure probably has little if any value, as several attempts to isolate *Brucella abortus* from the urine of positive cows have uniformly failed.

Guinea-pig inoculations and cultures have been made from the urine of 13 cows, most of which were strongly positive to the agglutination test for *Brucella abortus.* The bladders were secured at the time of slaughter, and 50 to 100 cc of urine centrifuged. The sediment and the film which appeared on the surface of the centrifuged

urine were inoculated into the peritoneal cavity of guinea pigs, and cooked-blood-agar plates were planted. In all cases the cultures and guinea pigs were negative for *Br. abortus*.

The inoculation of urine from human or caprine sources may give positive results. The occurrence of the *Brucella* organism in the urine of artificially inoculated guinea pigs has been reported several times, and will be dealt with later in this paper.

Blood.—Citrated or defibrinated blood may be inoculated into the peritoneal cavity of guinea pigs. Blood which has been allowed to clot is thoroughly ground in a sterile mortar, suspended in salt solution, and inoculated. The method devised by Boez and Robin[4] for successful blood cultures of various organisms might well be used for animal inoculation, as well as for culture seeding, in the attempted isolation of *Brucella abortus*. By the use of acid potassium sulfate to reduce the pH of the blood at the time of collection, the bactericidal action is destroyed.

Briefly, the technique as outlined by Boez and Robin is as follows: The blood is drawn into a sterile tube containing 2 cc of the following solution:

Sodium citrate	2.75 grams
Potassium acid sulfate	5.60 grams
Distilled water to make	100 cc

The pH of the blood after being citrated with the above solution is about 5.5, sufficiently low, according to Boez and Robin, to destroy the bactericidal property of the blood. Ten cc of the citrated blood is added to 100 cc of glucose broth, which has a pH 8.3. The final reaction will be found to be about pH 7.5.

Soule[19] has published the following technique: Cattle blood was cultured for *Brucella abortus* by adding 50 cc samples to 450 volumes of sterile glycerol infusion broth and subsequently incubating in air enriched with CO_2. The blood was diluted as soon as withdrawn and was not chemically treated or defibrinated to prevent clotting. At 2–4 day intervals, samples were removed and streaked on the surface of glycerol agar. He reports tests on over 5,000 ordinary herd-run cows. In the first series of tests agglutinins were present in the blood stream of 2,237 of the animals but only 299 gave positive blood cultures. In the second series agglutinins were present in 2,607 cases with 206 positive blood cultures. There were 40 positive blood cultures reported without concomitant blood agglutinins.

Feces.—Barger and Hayes[3] were able to demonstrate the presence of *Brucella abortus* in the feces of calves which had ingested

infected material, by diluting about 5 grams of fecal material with 20 cc of sterile salt solution. About 2 cc of the resulting suspension was inoculated intraperiatoneally into guinea pigs.

Amoss and Poston[1, 2] more recently have outlined a method by which the *Brucella* organisms in human feces may be concentrated by use of strong *Br. abortus* antiserum, and isolated on eosin-methylene-blue plates.

METHODS OF INJECTION

While the subcutaneous inoculation of guinea pigs with suspected material is the most generally used method for the detection of *Brucella abortus,* intraperitoneal inoculation has been used in this laboratory with considerable success. There are advantages and disadvantages connected with both methods. The test animals are better able to overcome a large number of contaminating organisms when the inoculation is subcutaneous. In the case of milk which contains large numbers of streptococci, this is of considerable importance. However, milk or other material sufficiently contaminated to cause the death of the guinea pig when inoculated intraperitoneally is a very poor sample to use for the detection of *Br. abortus* by any method. Carpenter and Boak[6] have shown that the *Br. abortus* organisms are no longer viable in cream when the acidity reaches pH 5.0 or lower. Badly contaminated milk samples are usually below this figure.

In the routine examination of the individual cows in a large dairy herd during the past two years, approximately 1,300 guinea pigs have been inoculated intraperitoneally with milk. The samples were usually inoculated about 8 hours after being drawn, but not infrequently 24 hours elapsed between collection and inoculation. In this group, 37 pigs succumbed to peritonitis due, probably, to the inoculum. Of these animals, 17 were in one lot of 30 which received milk brought to the laboratory during very warm weather, without ice. It is doubtful if this later group would have become infected with *Brucella abortus* even though the organism had been present at the time the samples were collected. The loss from intraperitoneal inoculation of properly collected and handled materials is relatively unimportant. An advantage which this method has over subcutaneous inoculation is the possibility of inoculating comparatively large quantities of material; 3 cc or more may be given intraperitoneally with no ill effect, whereas such amounts given subcutaneously at a single point of injection cause acute discomfort to the animal. This is of particular importance in the inoculation of milk or other materials containing few *Br. abortus* organisms.

OPTIMUM TIME FOR AUTOPSY

The procedure which probably varies most widely in the different laboratories is the length of time which is allowed to elapse between inoculation and autopsy. This period varies from one week to two or more months. Nelson[15] has reported a method by which *Brucella abortus* may be recovered from the guinea pig 5 days after inoculation. Carpenter[5] keeps the pigs for from 4 to 5 weeks and Gilbert, Coleman, and Groesbeck[10] in a report on some comparative tests, reached the conclusion that guinea pigs inoculated with material suspected of containing *Br. abortus* should not be killed before the end of the the fifth week. These tests were made with two lots of animals, one killed between the fourth and fifth week, and the other at about the end of the eighth week, but no data were given as to the advantages or disadvantages of slaughter at any interval between these two periods.

In this laboratory it has been the custom to adhere rather rigidly to a period of from 40 to 44 days between inoculation and slaughter. This interval has given consistently good results, and the correlation of agglutinins, macroscopic lesions, and cultures has been rather close in the majority of cases.

A small series of comparative tests, the results of which are shown in table 1, would seem to indicate that a period of 8 weeks is slightly superior to one of 6 weeks. However, the maintenance of a large number of inoculated pigs for 2 additional weeks has many disadvantages, and the number of positive cases obtained by the additional time is probably rather small. In cases of particular interest or importance, the inoculation of two animals with the same material is very desirable because of the possibility of individual resistance of the test animal and of loss by intercurrent diseases. When two animals are inoculated, one may be slaughtered at the end of 6 weeks and the other held for 8 weeks in case the first is negative.

AUTOPSY TECHNIQUE

The technique outlined below is given in detail because in this laboratory it has been found to be a simple, convenient, and successful method which can be used for making post-mortem examinations of large numbers of guinea pigs. As will be shown later, the presence of *Brucella abortus* in the spleen of an inoculated animal is frequently the only indication of infection. For this reason this technique of

autopsy has been developed with the primary object of procuring a culture from this organ free from contamination.

The guinea pig to be autopsied is placed in a Novy jar containing chloroform and left until respiration has ceased. In order to obtain blood for the agglutination test, it is preferable not to allow the animal to remain in the chloroform until the blood begins to clot. After the animal has been dipped in a weak solution of liquor cresolis compositus, it is fastened to a Carpenter[5] autopsy board (fig. 2) and an incision is made in the skin from the throat to a point somewhat posterior to the sternum, along the median ventral line. Another cut is made from the throat to near the right foot, and the

Fig. 2. Carpenter autopsy board for the post-mortem examination of guinea pigs.

triangular flap of skin thus formed is freed from the body wall, exposing the right axillary region and forming a pocket in which blood may collect when the axillary vessels are severed.

When the blood has been transferred to a tube, the skinning is completed and loose hair is destroyed by passing the flame of an inverted Bunsen burner several times over the entire body. By means of a small soldering iron the surface of the left side of the abdomen is thoroughly seared. The board is then placed in an inclined position by elevating and propping the side to the operator's right. With flamed scissors and forceps, a roughly semicircular incision is made in the abdominal wall, starting about 1 inch anterior to the anus, bearing to the right and about 1½ inches from the median line, and crossing back over and beyond the median line at the tip of the sternum. The resulting flap of tissue is folded over to the left, and the intestines may be grasped with forceps and pulled toward the

left. By this means, because of the inclined position of the board, the stomach and intestines are dislodged and the spleen exposed in a position convenient for examination and culture. The tip of the spleen is grasped with flamed forceps held in the left hand, while scissors are used to free the spleen. The tissue is transferred to a cooked-blood-agar slant by means of a stiff chrome-wire loop. It is desirable to obtain as large a piece of tissue as possible for seeding. In the case of a normal-sized spleen, the entire organ is used except for a small piece at the tip which is clipped off in order to produce a rupture in the capsule. In the case of enlarged spleens, a piece of tissue approximately equal to a normal spleen is used.

After the culture has been made, the thoracic cavity may be opened and the animal examined for other lesions of brucelliasis.

All spleen cultures are incubated in 10 per cent carbon dioxide for 4 to 6 days; then the slants are examined and if no growth is visible, the spleen tissue is crushed and spread over the surface of the slant with a stiff wire loop. Frequently after further incubation, cultures are obtained by this method which would be overlooked if the tissue had been left undisturbed. After about 21 days of incubation, tubes showing no growth are discarded.

Transplants are made from all cultures and the resulting growth is suspended and used as agglutinating fluid and tested with known positive and negative *Brucella abortus* antiserum.

OBSERVATIONS ON THREE CRITERIA OF INFECTION IN GUINEA PIGS

Agglutinin Production in Infected Guinea Pigs.—Little correlation is found between the number of organisms inoculated and the agglutinin titre in the blood of guinea pigs at the end of 6 weeks. This refers only to animals inoculated with naturally infected milk, the bacterial count of which has been determined by counts on gentian-violet cooked-blood agar. The blood-serum titre of the infected animals, at the time of slaughter, varies from negative at a dilution of 1 to 25 to positive at 1 to 6,400, with the majority positive within the range between dilutions of 1 to 400 and those of 1 to 1,600. The number of organisms found in naturally infected milk is relatively small, and the number inoculated in 2 cc of cream and sediment rarely exceeds 10,000.

Extensive lesions, typical of brucelliasis in a test animal, are usually accompanied by demonstrable agglutinins in the blood serum, but the extent of the lesions is no indication as to the titre of the *Brucella* agglutinins. Occasionally slight but definite lesions are

found in animals whose blood serum fails to cause agglutination of *Br. abortus* antigen even at a dilution of 1 to 25. A number of animals have been encountered with very extensive lesions whose blood serum had a titre of only 1 to 50 or 1 to 100.

In an appreciable percentage of cases, guinea pigs will be found whose blood serum is negative at a dilution of 1 to 25 but whose spleen yields *Brucella abortus* when cultured. These animals may show slight macroscopic lesions, but are usually normal in appearance. In compiling the results of 1,214 guinea pigs inoculated with suspected material, it was found that 438 cultures of *Br. abortus* were obtained from the spleens. Of this number, 28, or slightly over 6 per cent, were obtained from guinea pigs whose blood showed no agglutination at a dilution of 1 to 25 or over.

In the light of these findings, the rather widespread practice of keeping test animals for an indefinite time and eventually discarding them as negative if the agglutination test performed with their blood is repeatedly negative, seems open to considerable objection. How many of the 28 guinea pigs mentioned above would have eventually exhibited agglutinins, it is of course impossible to determine, but it seems probable that some of them would have been able to overcome the infection without any agglutinin production. Still other animals may be physiologically unable to produce antibodies regardless of the course of infection, and therefore would be included in the negative group if agglutinin production alone were depended upon. Nonvirulent variants of *Br. abortus* which produce no agglutinins have also been isolated from the spleens of guinea pigs at least 9 weeks after inoculation.

Of 427 animals with an agglutinin titre of 1 to 25 or over, cultures of *Brucella abortus* were not obtained from 17, or 4 per cent. Eight of these failures were due to contamination and we feel that the remainder could be explained by some failure in technique. In view of these results, it seems highly probable that *Brucella abortus* can be isolated from the spleen of any guinea pig whose agglutinin titre for this organism is 1 to 25 or higher, if the culture is made 6 weeks after inoculation.

Lesions in Infected Guinea Pigs.—In the routine examination of guinea pigs for lesions of brucelliasis 6 weeks after inoculation, organs which will be found to be most frequently involved are the spleen, the liver, the male genital organs, the precrural, sublumbar, and inguinal lymph nodes, the lungs, and the leg joints. For a detailed and complete description of lesions, the reader is referred to the works of Fabyan,[8] Schroeder and Cotton,[16] or Jaffe.[14] However, it must be borne in mind that the lesions described by these authors

are, in most cases, the results of infections with massive doses followed by an interval of several months before autopsy, and comparable lesions are not to be expected when test animals are slaughtered 6 weeks after inoculation.

Macroscopically the spleen may be enlarged slightly or may be enlarged up to six times its normal size. The surface is usually nodular (fig. 3), but in cases of an acute nature, the spleen may be

Fig. 3. Liver and spleen of guinea pig killed 6 weeks after intraperitoneal inoculation with milk containing *Brucella abortus.*

smooth. The nodules in the early stages of their development are hemorrhagic, later becoming encapsulated, grey, discrete, and may have a necrotic center.

Just beneath the capsule on the surface of the liver, small grey glistening nodules may usually be found. These nodules range in size from 0.5 to 2.0 mm in diameter, are discrete, and may or may not have an opaque center. In exceptional cases the entire liver may be studded with the nodules, but usually from 10 to 50 occur (fig. 3).

Macroscopic lesions in the female genital organs are so rare that they are of no importance for routine diagnosis. The reverse is true, however, in the case of the male organs. In most cases of intraperitoneal inoculation of male guinea pigs, lesions may be found in the testicles proper, in the epididymis, or in the walls of the sac surrounding the organ. Adhesions of the testicles or epididymis to the sac are frequent. Abscesses containing creamy, yellow, or white pus are occasionally found in the testicle proper (fig. 4), but more frequently in the epididymis (fig. 5). The tubules of the epididymis

become enlarged and may be disintegrated sufficiently so that they do not appear sharp in outline as do the tubes in a normal organ. Abscess formation may occasionally be found in the cremaster muscles with no macroscopic change in the epididymus. Atrophy of one or both testicles also occurs, with or without abscess formation.

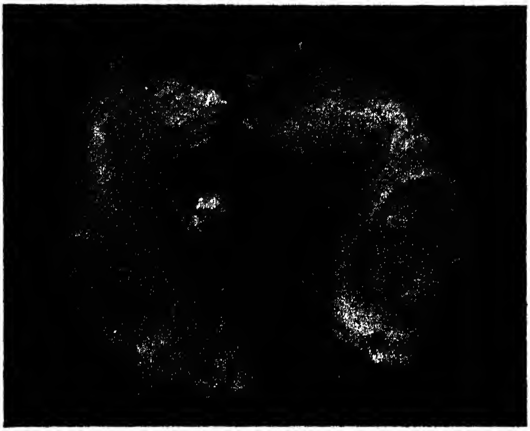

Fig. 4. Testes of guinea pig killed 6 weeks after intraperitoneal inoculation with milk containing *Brucella abortus*. Note the abscess formation in the distal portions of the testes proper.

Fig. 5. Testes of guinea pig killed 6 weeks after intraperitoneal inoculation with milk containing *Brucella abortus*. Note abscesses in epididymes.

Noticeable enlargement of the sublumbar lymph nodes almost invariably accompanies testicular involvment. This increase in size usually varies from two to six times normal. The nodes are often hyperemic and occasionally definitely hemorrhagic. Abscess formation is rather rare in the lymph nodes of animals that are inoculated with small numbers of bovine *Brucella abortus* and kept only 6 weeks

before slaughter. The precrural and inguinal lymph nodes often show alteration similar to that found in the sublumbars. Macroscopic changes in other lymph nodes of the body are relatively rare, although occasionally enlargement of axillary, mesenteric, and bronchial nodes is noted.

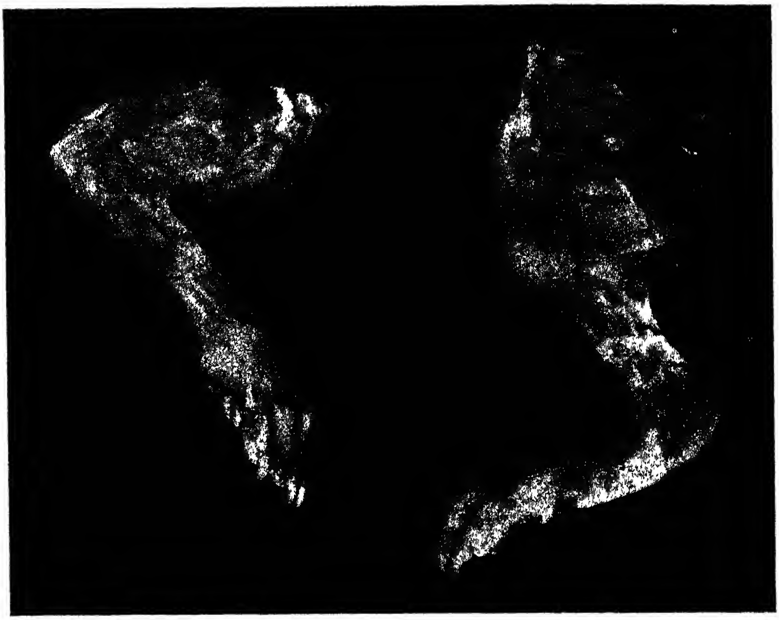

Fig. 6. Joint lesions in guinea pigs, produced by the injection of massive doses of *Brucella abortus*.

In a rather small percentage of cases, clear glassy irregularly shaped areas are found just beneath the pleura. These vary from 1 to 5 mm in diameter. They usually show an opaque center. When the lesions in the lungs are large or numerous, some enlargement of the bronchial lymph nodes occurs.

Lesions of the joints are extremely rare in animals inoculated with bovine tissues and materials, and slaughtered at the end of 6 weeks. With massive infecting doses such as cultures or infected utero-chorionic exudate, swollen carpel and tarsal joints occur rather frequently (fig. 6).

In addition to the previously mentioned manifestations, other less common lesions may from time to time be encountered. In this group would be included an abscess 1 cm in diameter filled with creamy pus, on the wall of the urinary bladder (fig. 7), which upon culture yielded only *Brucella abortus;* subcutaneous or intramuscular ab-

scesses in the abdominal wall at the point of inoculation which may be caused by *Br. abortus;* and a large subcutaneous abscess behind the ear, open to the outside, which also yielded *Br. abortus.*

In the examination of large numbers of guinea pigs for lesions of brucelliasis, it will be found that those outlined above may appear singly or in almost any combination; however, the spleen, liver, and

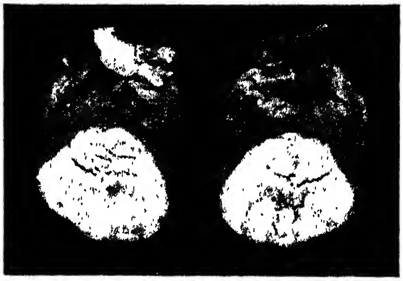

Fig. 7. Abscess on wall of urinary bladder of a guinea p'g. This animal had been inoculated with milk 6 weeks previous to the time of autopsy.

testicle lesions are ordinarily rather uniform as to extent and occurrence. The liver lesions are the most characteristic of all the lesions described. It is rare indeed to find typical small grey translucent nodules on the surface of the liver without finding *Brucella abortus* agglutinins in the blood or obtaining *Br. abortus* in cultures made from the spleen. On the other hand, the absence of liver lesions is not necessarily indicative of freedom from *Brucella* infection.

The relation of lesions to agglutinins and to positive cultures is rather close. However, in a total of 516 guinea pigs which showed

TABLE 4

OCCURRENCE OF AGGLUTININS, LESIONS, AND POSITIVE SPLEEN CULTURES IN GUINEA PIGS INOCULATED WITH MILK NATURALLY INFECTED WITH BRUCELLA ABORTUS AND SLAUGHTERED AT THE END OF 6 WEEKS

Agglutinins in blood	Lesions	Cultures	Number of animals	Percentage of total
+	+	+	427	82.7
–	+	+	5	1.0
+	–	+	56	10.8
–	–	+	22	4.3
+	+	–	1	0.2
–	+	–	4*	0.8
+	–	–	1	0.2
Total animals			516	100.0

* These guinea pigs showed lesions which were macroscopically indistinguishable from those produced by infection with *Brucella abortus*, but which might have been due to other causes.

evidence of infection, the correlation shown in table 4 was observed. From this table it will also be found that 98.8 per cent yielded positive spleen cultures, 93.8 per cent showed agglutinins in their blood at a dilution of 1 to 25 or over, and 84.7 per cent showed macroscopic lesions.

Cultures from Infected Guinea Pigs.—

1. Spleen cultures: Most workers have found the spleen to be the best organ for use in obtaining cultures of *Brucella abortus*. Observations in this laboratory in the past four years on some 3,000 guinea pigs inoculated with materials suspected of containing *Br. abortus* have amply borne out this conclusion. As stated above, it rarely occurs that one fails to obtain a culture from the spleen of an animal that manifests any other indication of infection. This holds true for guinea pigs whose spleens show no macroscopic lesions, as well as for those whose spleens show gross pathological changes.

2. Urine cultures: In order to determine the occurrence of *Brucella abortus* in the blood and urine of infected guinea pigs, a series of cultures were made from the heart blood and from the urine of animals showing macroscopic lesions of brucelliasis.

The cultures from the urinary bladder were made by thrusting a Pasteur pipette through the wall after the bladder had been thoroughly seared to avoid contamination with *Brucella abortus* from the peritoneal cavity. One-half to 2 cc was obtained in most cases, but only a drop or two in a few instances. The urine was transferred to cooked-blood-agar slants and the slant tipped once or twice in order to insure distribution over the surface. The cultures were incubated in 10 per cent carbon dioxide.

Cultures were made from a total of 106 infected animals and *Brucella abortus* was recovered from 24, or 22.6 per cent of these. Seventy-nine of the cultures showed no growth, 2 gave pure cultures of a *Streptococcus*, and 1 was overgrown with an air contaminant. Spleen cultures were obtained from all these animals.

The difference between the percentage of positive urine cultures in the males and in the females was marked. The sex was recorded in only 93 of the 106 animals whose urine was cultured. Of these, 50 were females and 43 were males. Five of the females (10 per cent) yielded positive cultures, whereas 15 (34.8 per cent) of the urine cultures from the males were positive. This difference is undoubtedly due to the frequency with which the male genital organs are infected, and it may be assumed that in many cases the organisms are carried to the bladder from broken-down testicular lesion. However, one case (fig. 7) has been observed of a definite infection in the bladder wall.

3. Blood cultures: Heart-blood cultures were made in a manner similar to that employed for securing urine cultures. In the case of blood cultures, however, it is important to stir the clotted blood 4 to 6 days after the initial seeding. A considerable number of the cultures which proved positive after the thorough stirring would have been discarded as sterile if the clot had not been broken up. Close inspection of the tube is necessary to avoid discarding cultures containing a small amount of growth. Several cases were encountered in which the only evidence of growth was a thin grey film between the tube and the butt of the slant. While most of the positive cultures showed very few colonies, several were encountered in which, before stirring, the surface of the slant was completely studded with colonies.

In cultures made from the heart blood of 126 infected guinea pigs, *Brucella abortus* was recovered from 60, or 47.6 per cent. There was no significant difference between the number of positive cultures obtained from the male and the female guinea pigs. Of the 117 animals whose sex was recorded, 66 were female and 51 were male. Positive cultures were obtained from 33, or 50 per cent, of the females, and from 24, or 47 per cent, of the males.

SUMMARY

A comparison of direct-culture methods with guinea-pig inoculations for the isolation of *Brucella abortus* from milk has been made. Guinea-pig inoculations have proved much more efficient.

The technique for the isolation of *Brucella abortus* by guinea-pig inoculation and by culture methods is given in detail.

An attempt has been made to determine the reliability of the three common criteria of infection in artificially inoculated guinea pigs; namely, agglutinin production, lesions, and spleen cultures. The relation of positive spleen cultures, agglutination titers over 1 to 25, and macroscopic lesions, in a total of 516 guinea pigs showing some evidence of infection, was 98.8 per cent, 93.8 per cent, and 84.7 per cent, respectively. For this reason the spleen-culture indication is considered the most reliable of the three.

The incidence of *Brucella abortus* in the urine and blood of guinea pigs 6 weeks after inoculation with infected material has been observed. This organism was recovered in cultures of the urine in 22.6 per cent of 106 guinea pigs known to be infected, and the frequency of the organism in the urine of males was over three times that in the urine of females. *Br. abortus* was also obtained in cultures from the blood in 47.6 per cent of 126 guinea pigs known to be infected. There was no significant difference between the number of positive blood cultures obtained from the male and the female guinea pigs.

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PHYSICAL AND CHEMICAL CHANGES IN THE RIPENING OF DECIDUOUS FRUITS

F. W. ALLEN[1]

INTRODUCTION

Interest on the part of many growers, shippers and others in determining the most satisfactory degree of maturity of deciduous fruits for eastern shipment has led to considerable investigational work in this field. Two publications, previously issued by the California Agricultural Experiment Station[2, 3] give the results of maturity studies with plums and the Bartlett pear. Recommendations were included for the harvesting of these fruits. The purpose of this publication is not to present additional maturity standards but rather to make available additional data on the ripening changes which take place in the above-mentioned fruits and to present similar data on apricots, peaches, and apples. The discussion includes the more familiar physical and chemical changes which occur during the period of fruit maturity and ripening, together with the results secured in the ripening of fruit by the use of ethylene gas.

INCREASE IN SIZE

Complete growth records, such as determined by Hendrickson and Veihmeyer[9] for peaches, and by Lilleland[20] for apricots, were considered nonessential to a study of ripening changes. However, as certain ripening changes take place previous to the complete sizing of the fruit, growth measurements were taken during the later stages of fruit development. Increase in size was determined by taking

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circumference measurements of from 20 to 40 individual fruits from several trees. During the season of 1924, increase in size was determined by measuring representative fruits of a given color stage as they were harvested. During 1925 and 1926 the individual fruits were tagged and measured on the tree at frequent intervals during the period of greatest color change. In presenting the results, emphasis is placed on the relative increase in volume rather than on actual gain as ascertained by measurement.

Plums.—The attention given the trees, the water content of the soil, and the size of the crop on the tree are all important factors influencing size changes. As pointed out in a previous publication[2] instances were found where plums made practically no increase in size after losing their original green color. The greatest gain over a period of ten to fifteen days immediately preceding or during the period of harvest was found to be 4.5 per cent in volume per day, while in most instances the daily increase varied between 1.5 and 2 per cent. Increase in size of plums was usually slightly greater in the early stages of ripening—from the time the fruit changed from a decided green to slight pink or blue—than it was in the later color stages. Growth does not altogether cease until the fruit attains or closely approaches its full color—a greater degree of maturity than most varieties are allowed to reach before being harvested for eastern shipment. Late-ripening varieties made a smaller daily gain in size than early sorts. Early varieties, however, are the ones most often picked prematurely and a five or six days' delay in harvesting would in many instances not only result in fruit of more desirable size but in an approximate 10 per cent increase in the number of crates produced.

Peaches.—The measurements and observations shown in table 1 taken on the growth and color of peaches from several orchards in Placer County from the time of 'breaking color' until completely colored, show in most instances even a more marked increase in growth during their late development than was found with plums. Peaches are characterized by a decided 'filling out' or 'swelling' after they begin to show some of their characteristic color. Owing to the firmness of the flesh and the fact that they are primarily used locally for canning, clingstone varieties are usually permitted to attain practically their full growth before harvesting. Freestone varieties, grown for shipment to the eastern markets as fresh fruit, are usually harvested while still somewhat immature and undersized. It is recognized that soft fruits such as peaches must be harvested sufficiently early to withstand the necessary physical handling and the delay in transit. On the other hand, the possibility of attaining better

TABLE 1

INCREASE IN SIZE AND CHANGE IN COLOR OF PEACHES TEN DAYS TO TWO WEEKS BEFORE PICKING; PLACER COUNTY, 1924–1926

Variety	Date of measurement	Color stage when picked	Average circumference, centimeters	Average volume,* cubic centimeters	Average daily increase in volume between measurements, cubic centimeters
Early Crawford	July 14, 1925	Greenish yellow, slight blush	17.6	92.1	....
	July 20, 1925	Full yellow, good blush	18.8	112.2	3.35
	July 25, 1925	Full yellow, good blush	20.1	137.2	5.00
Early Crawford	July 1, 1926	Light green, slight blush	15.8	66 6	....
	July 7, 1926	Greenish yellow, slight blush	16.5	75.9	4.81
	July 12, 1926	Yellow, ⅛ to ½ red	19 8	131.1	7.74
Elberta	July 14, 1924	Green to slight greenish yellow	17.1	84.5	....
	July 22, 1924	Greenish yellow, slight blush	18 9	114.0	4.21
	July 29, 1924	Full yellow, ½ red	19.6	127.2	1.89
Elberta	July 18, 1924	Green with slight blush	16.4	74.5	....
	July 30, 1924	Greenish yellow, ¼ to ⅓ blush	18.7	110 5	3.66
	August 1, 1924	Greenish yellow, ⅓ blush	19.5	125.2	....
Elberta	July 20, 1925	Greenish yellow	18.2	101.8	....
	July 30, 1925	Light yellow, slight blush	21.0	156.4	5.46
	August 6, 1925	Full yellow, good blush	22.4	189.8	4.77
Elberta	July 14, 1926	Green, slight blush	16.9	81.5	....
	July 23, 1926	Greenish yellow, slight to ⅓ red	18.1	100.0	2.05
	July 27, 1926	Yellow, slight to ⅓ red	18.5	106 9	1.72
Phillips Cling	August 17, 1925	Greenish yellow to yellow	18.9	114.0	....
	August 25, 1925	Yellow, ¼ to ½ red blush	21.5	167.9	6.73
	September 2, 1925	Golden yellow, ¼ to ¾ blush	22.5	192 4	3.06
Triumph	June 5, 1926	Green, ¼ to ½ red	15.4	61.7	....
	June 9, 1926	Yellowish green, ½ to ¾ red	16.3	73.1	2.85
	June 16, 1926	Full dark red	17.6	92.0	2.70
Tuskena	July 3, 1925	Greenish yellow, slight blush	18.2	101.8	....
	July 9, 1925	Full yellow, ¼ blush	20.5	145.5	7.29
	July 13, 1925	Full yellow, ½ red	21.8	175.0	7.37
Tuskena	July 17, 1925	Greenish yellow, slight blush	17.8	95.2	....
	July 20, 1925	Full yellow, ¼ to ¾ red	18.6	108.8	4.53
	July 27, 1925	Full yellowish red	20.0	135.1	3.75
Tuskena	July 6, 1926	Greenish yellow, slight to ½ red	16.2	71.8	....
	July 9, 1926	Yellow, ½ to ⅔ red	17.3	87.4	5.20
	July 13, 1926	Yellow, ¾ to full red	18.5	106.9	4.87
	July 16, 1926	Yellow, mostly full red	19.2	119.5	4.20

* In most instances it was necessary to compute the volume from the diameter or circumference of the fruit. $\left(\text{Vol.} = 1/6\pi D^3 \text{ or } \frac{C^3}{6\pi^2}.\right)$ For comparative purposes the fruit was assumed to be spherical and to show little change in form as it developed.

size and better quality in such fruits should encourage growers to avoid 'rushing the season.'

Pears.—In the early sections of the state, commercial practice dictates that the harvesting of Bartlett pears begin as soon as the fruit attains a diameter of 2¼ inches (5.7 centimeters, or a circumference of 18 centimeters). Unless produced on weak, overloaded

TABLE 2

INCREASE IN SIZE AND CHANGE IN COLOR OF BARTLETT PEARS PREVIOUS TO AND DURING THE PERIOD OF COMMERCIAL HARVEST; PLACER COUNTY, 1924–1926

Orchard No.	Date of measurement	Color stage when picked	Average circumference, centimeters	Average volume, cubic centimeters	Average daily increase in volume between measurements, cubic centimeters
1	July 18, 1924	Green	19 9	133.1	....
	*July 30, 1924	Yellowish green, slight blush	21 3	163.2	2 50
	August 6, 1924	Greenish yellow	21.6	170 2	1.00
2	July 18, 1924	Green	19.7	129 1	......
	*July 30, 1924	Yellowish green, slight blush	20.6	147 6	1.54
	August 6, 1924	Yellowish green, slight blush	21.6	170 2	3.23
	August 15, 1924	Greenish yellow, firm ripe	22.5	192.4	2.46
3	July 10, 1925	Green	18 9	114.0	....
	*July 20, 1925	Green to yellowish green	19.7	129.1	1 51
	July 30, 1925	Greenish yellow, slight blush	20.7	149.8	2.07
4	June 11, 1926	Green	15.1	58.1	....
	June 18, 1926	Green to slight yellowish green	16.3	73.1	2.14
	June 25, 1926	Yellowish green	17.1	84 4	1.61
	*July 2, 1926	Yellowish green	17.6	92.0	0.95
	July 9, 1926	Greenish yellow	17 8	95.2	0 45
	July 16, 1926	Greenish yellow to light yellow	18.0	98.5	0.47

* Beginning of commercial harvest.

trees or those growing under drought conditions, fruit of this size is harvested at a great sacrifice in yield. Under favorable growing conditions very material and consistent gains in size are made until the fruit is considered too mature for commercial shipment to distant markets. Table 2 illustrates some of the measurements taken in Placer County during 1924–1926.

Apples.—Measurements taken on Gravenstein apples in two large commercial orchards of the Sebastopol district show that this fruit also continues to increase in size beyond the time when usually picked commercially. As this variety is the first boxed apple of the season

to appear on the markets, harvesting is practically at its height when the fruit is making rapid gain in size. Initial pickings are usually made by July 1. Table 3 illustrates the growth which is being made by the fruit at this time.

TABLE 3

INCREASE IN SIZE AND CHANGE IN COLOR OF GRAVENSTEIN APPLES PREVIOUS TO AND DURING THE PERIOD OF COMMERCIAL HARVEST; SEBASTOPOL, 1926

Orchard No.	Date of measurement	Color stage when picked	Average circumference, centimeters	Average volume, cubic centimeters	Average daily increase in volume between measurements, cubic centimeters
1	June 17	Yellowish green	20.5	145.5	....
	June 24	Yellowish green to greenish yellow	21.7	172.6	3.87
	July 1	Yellowish green to greenish yellow	23 5	219.2	5.82
	July 7	Yellowish green to greenish yellow	24 0	233.5	2 38
	July 21	Greenish yellow to light yellow	24 5	248.4	1.06
2	June 18	Yellowish green	20.5	145.5	....
	June 25	Yellowish green to greenish yellow	22.0	179.9	4.91
	July 2	Yellowish green to greenish yellow	23.0	205.5	3.20
	July 8	Yellowish green to greenish yellow	24.0	233.5	4.66

DEVELOPMENT OF COLOR

Color changes in deciduous fruits may be divided into two classes, those affecting the ground or undercolor, and those affecting the development of the red, blue, and other overcolors. The former, brought about by the loss of chlorophyll and the unmasking and development of the yellow pigment or carotin material in the plastids of the cell is of major importance to commercial fruit growers in determining maturity. This 'breaking' of the original green color takes place independently of sunlight although influenced by it.

The overcolors characterizing the fully ripened fruit are usually ascribed to some of the anthocyanin pigments dissolved in the cell sap, or perhaps to a combination of these with the carotin-like materials in the plastids. Strawberries, blackberries, grapes, cherries, and plums are usually considered able to develop their mature color, though perhaps to a less degree, when sunlight is excluded. Overholser,[(31)] however, reports that owing to delayed maturity, blackberries, cherries, and plums of the *Prunus americana* species colored only in part when they were enclosed in black bags. Smith and Smith[(34)] report that Elberta peaches thus enclosed developed a higher

carotinoid content than unbagged fruit, but that the reverse was true with the Humboldt nectarine and the Royal apricot. That some of the more important varieties of *Prunus domestica* and *Prunus salicina* will color satisfactorily without light, has been shown in a previous publication.[2] Apples, peaches, nectarines, pears, and apricots are generally regarded as requiring sunlight for the development of their red color. Overholser found that the Williams apple developed some slight color when ripened under black cloth bags.

Not only is light essential for the coloring of most of the more important tree fruits but Magness,[26] and also Fletcher,[8] point out the particular value of the ultraviolet rays of light. They also found that red color intensity is closely associated with the carbohydrate or sugar content of the fruit. In fact, Magness, regulating the potential carbohydrate food supply of the individual fruits by controlling the leaf area, states that the first determining factor in the coloring of apples is chemical composition rather than weather and light conditions. Fletcher reports securing an increase in color through the addition of sugar to the soil. That there is also some correlation between color development of plums and the soluble solids in the juice and sugar content of the fruit is shown by the data in table 19. Since, however, it is difficult to control accurately the chemical composition of the different specimens it cannot be stated definitely whether the excess sugar was fundamental to, or simply accompanied, the coloring. In contrast to an increase in color development with a high carbohydrate supply it is generally recognized that nitrogen causes a decrease in color, probably due to shade.

The commercial orchardist recognizes that local climatic conditions and the variety in question are also important factors influencing the amount and rapidity of coloring. Stone fruits, grown under interior valley and foothill conditions, undergo rapid changes in color from ten days to two weeks before becoming ripe. The red color of cherries, the yellow of apricots, and the red and blue color of plums quickly follow the earlier changes in the ground color. The blush on the exposed side of peaches may even precede any appreciable change in ground color.

Plums.—The varieties of European (*Prunus domestica*) and Japanese (*P. salicina*) plums grown in California are numerous, and the different varieties show differences in the amount and rate of coloring. Kelsey plums show almost no color change at any time. Wickson and Formosa change only to a yellow or slight pink, while Santa Rosa, Tragedy, Diamond, and President should, when in prime eating condition, be of very dark red, blue, or purple color. Table 4

TABLE 4

DEVELOPMENT OF COLOR IN PLUMS PREVIOUS TO AND DURING THE PERIOD OF COMMERCIAL HARVEST; PLACER COUNTY, 1926

Variety	Date	Color stage
Beauty	May 26	Green
	May 30	Straw tip to light straw
	*June 2	Light pink to pink tip
	June 8	¼ to ½ red
Formosa	June 3	Straw tip to light straw
	*June 8	Full straw to yellow..
	June 16	Slight to ⅓ pink
	June 18	½ to ¾ pink
Santa Rosa	June 5	Green
	June 11	Straw tip to straw
	June 16	Pink tip to ¼ pink
	*June 20	¾ to full red
	June 23	Full dark red
Climax	June 5	Green
	June 10	Straw tip to light straw
	June 15	Full straw
	*June 20	Pink tip to slight pink
	June 22	¾ to full red
Wickson	June 8	Green
	June 16	Straw tip
	June 23	Light straw
	*July 1	Full straw to yellow
Burbank	June 17	Green to light straw
	June 24	Full straw to trace pink
	*June 30	Slight pink to ¼ red
	July 6	Full yellow to ¾ red
Duarte	June 23	Green to straw tip
	June 30	Pink tip to ⅓ light pink
	*July 6	½ pink to pink
	July 12	Full red
Diamond	June 30	Trace blue to blue tip
	*July 7	⅓ to ¾ blue
	July 12	Full blue
	July 16	Full dark blue
Giant	July 7	Straw to trace pink
	July 12	Slight to ½ pink
	*July 23	¾ to full pink
	July 28	¾ to full red
President	July 23	Slight to ⅓ purple
	July 27	½ to ¾ purple
	*July 30	Full purple

* Approximate time of first commercial pickings.

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	June 10	Straw tip to light straw
	June 15	Full straw
	*June 20	Pink tip to slight pink
	June 22	¾ to full red
Wickson	June 8	Green
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	June 23	Light straw
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Burbank	June 17	Green to light straw
	June 24	Full straw to trace pink
	*June 30	Slight pink to ¼ red
	July 6	Full yellow to ¾ red
Duarte	June 23	Green to straw tip
	June 30	Pink tip to ⅛ light pink
	*July 6	½ pink to pink
	July 12	Full red
Diamond	June 30	Trace blue to blue tip
	*July 7	⅛ to ¾ blue
	July 12	Full blue
	July 16	Full dark blue
Giant	July 7	Straw to trace pink
	July 12	Slight to ½ pink
	*July 23	¾ to full pink
	July 28	¾ to full red
President	July 23	Slight to ⅛ purple
	July 27	½ to ¾ purple
	*July 30	Full purple

* Approximate time of first commercial pickings.

illustrates the color changes of marked specimens of some of these varieties previous to their removal from the tree. Seasonal variations will influence coloring in different years but these data are comparable and are typical of the color changes in most seasons.

Plums are a striking example of a fruit, which, unless picked before there is any change in the original ground color, will assume its full color after harvesting. As shown previously[2] the rate of development of red or blue color depends upon the temperature to which the fruit is exposed. Under ordinary air temperatures at harvest time, a week may be sufficient to attain full color. Under temperatures existing in the top of refrigerator cars (50°–55° F) the fruit picked with only very slight color will be well colored on arrival in the eastern markets some ten days later. Similar fruit carried in the cooler parts of the car where the temperature is 10° to 15° lower will show only slight changes in color.

Apricots and Peaches.—Early and midseason apricots are in prime eating condition as soon as they attain a medium yellow color. Tilton, a late variety, usually assumes, under California valley conditions, a rich golden yellow a week or ten days previous to becoming soft. Similar differences in color are noted between such varieties of peaches as the Early Crawford or the Elberta and the later varieties such as Tuskena and Phillips Cling. The time required for the yellow or red color to develop to a maximum consistent with good shipping conditions will vary, as shown by table 1, from ten to fifteen days. Apricots and peaches normally develop their yellow color rapidly after harvesting, and as with plums, the rapidity with which this occurs, again is controlled by temperature. Characteristic color changes for five varieties of peaches held for 10 days under temperatures usually existing in the bottom and top half of the load in refrigerator cars are shown in table 5. While it is generally conceded that these fruits are unable to take on additional red color under storage or transit conditions, the red color of the Tuskena peach becomes much more noticeable with the disappearance of the green ground color.

Pears.—Observations on the handling of pears show that both the variety and the location are factors influencing the amount and rate of coloring. Bartletts from the Sierra foothill areas and those grown in other sections of higher altitudes may possess a distinct yellow color before harvesting. In contrast, fruit from the Santa Clara Valley is picked while of a distinctly green color and loses little of its green color until the flesh has become overripe. Between these extremes, pears from the Sacramento River area picked when of a light green color, will, when subsequently exposed to room tempera-

TABLE 5

Changes in Color of Peaches Under Refrigerator Car Temperatures

Variety	Color when picked	Color after 10 days at 43° F	Color after 10 days at 52° F
Early Crawford	Light green, faint blush	Yellowish green, faint blush	Greenish yellow to light yellow, faint blush
	Greenish white, slight blush	Greenish yellow to light yellow, slight blush	Full yellow, slight blush
	Light yellow to yellow, ¼ blush	Full yellow, ¼ blush	Full yellow, ¼ blush
	Full yellow, ¼ to ½ blush	Deep yellow, ¼ to ½ blush	Deep yellow, ¼ to ½ blush
Elberta	Light green to yellow green	Yellowish green	Greenish yellow
	Greenish yellow	Greenish yellow	Yellow
	Greenish yellow to yellow, slight blush	Greenish yellow to light yellow, slight blush	Full yellow, slight blush
	Light yellow to full yellow, ⅓ blush	Full yellow, ⅓ blush	Full yellow, ⅓ blush
Tuskena	Greenish white, slight blush	Greenish yellow, slight blush	Yellow, slight blush
	Yellow, ⅓ blush	Yellow, ⅓ blush	Yellow to reddish yellow, ⅓ blush
	Yellow to ½ dark red	Reddish yellow to ½ dark red	Reddish yellow to ½ dark red
Triumph	Greenish white, slight red	Greenish yellow, slight red	Full yellow, distinct blush
	Yellowish green, ⅓ red	Greenish yellow, ⅓ red	Light yellow, ⅓ red
	Yellowish green, ½ red	Greenish yellow, ½ red	Yellow, ½ red
	Yellow, ½ red	Yellow, ½ red	Full yellow, ½ red
Phillips Cling	Greenish yellow to yellow	Greenish yellow to yellow	Full yellow
	Yellow, ¼ to ½ blush	Yellow, ¼ to ½ blush	Golden yellow, ¼ to ½ blush

tures of 70° to 80° F, assume an attractive yellow in 5 to 8 days. If stored under transit temperatures of 50° for 10 days the same degree of coloring will develop in 2 to 4 days after being exposed to the above room temperatures. Fruit held continuously at 32° will gradually assume its characteristic color.

Late varieties of pears, grown primarily in the Santa Clara Valley, are usually harvested after only a very slight change in color. Held at 70° F following harvest, Comice will show a color change in

TABLE 6

CHANGES IN COLOR OF GRAVENSTEIN APPLES UNDER REFRIGERATOR CAR AND ROOM TEMPERATURES; 1930

Orchard No.	Date picked	Color*				
		When picked	After 12 days at 43° F	Followed by 1 week at 70° F	After 12 days at 52° F	Followed by 1 week at 70° F
1	June 9	1-2	1½-2	4+	1½-2	4+
	June 25	2-3	3	4+	3	4+
	July 8	2-2½	2½-3	4+	3	4+
2	June 11	1-2	1½-2	3	2	3
	June 25	2-2½	2½	2-3	2½	2½
	July 8	2-3	2½-3	3½-4	3	3-4+
3	June 11	1-2	1½-2	2-3	2	4+
	June 25	2-2½	2½	4	3½	4+
	July 8	2½	2½	3½-4	3½-4+	....
4	June 25	2-2½	2½	4	3	4+
	July 8	2-3	3	4+	3-4	4+

* Color was determined by comparison with the standard color chart used by the California State Department of Agriculture where the numerical values indicate: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow.

5 to 9 days similar to Bartlett. Most other late varieties show a slower change after picking. Very late varieties such as Winter Nelis, Glou Morceau, Easter Buerre, and P. Barry require from 8 to 20 days to show any material change in color, even after a period of several months in storage. Efforts to ripen these varieties immediately after harvesting most frequently result in the fruit shriveling and becoming tough without any appreciable change in color. Winter Nelis pears, however, have been successfully ripened in a period of four weeks, when held under a temperature of 50°.

Apples.—Since the leading varieties of apples grown in California are of the green or yellow sorts—Yellow Newtown, Yellow Bellflower, and Gravenstein—color changes previous to harvesting are limited

primarily to the initial breaking of the ground color. Yellow Bellflower, harvested when of a light to yellowish green color, quickly changes to a light yellow under ripening temperatures. The Yellow Newtown—some of the fruit being light green and some light yellow when harvested—increases its color very little under storage temperatures. The Gravenstein may be allowed to develop some of its characteristic striping; however, early picking in order to place this variety on the market in the forepart of July largely precludes the development of this overcolor. The fruit is therefore harvested when of a light green to yellowish green and when fully ripe is of a light yellow with the better-colored specimens showing some red streaks. Table 6 gives the rapidity of these changes under refrigerator car temperatures while in transit, and under ordinary room temperatures after arrival at destination. Temperatures of 43° and 52° F represent the average temperatures usually existing in the bottom and top halves of the load.

SOFTENING OF THE FLESH

Softening of the flesh has long been recognized as one of the principal ripening changes in deciduous fruits but it appears that only recently were any attempts made to measure it. Several years before publishing the fact, Morris[28] conceived the idea of measuring by mechanical means the rate of softening of apples in storage. Later, the simple device used by Morris was improved by Murneek[29] and also by Magness and Taylor.[23] With these two instruments, or pressure testers, together with still more recent modifications, a large amount of investigational work on fruit softening has been done by Magness and his associates,[22, 24, 25] by Hartman,[11, 12, 13, 14, 15] Morris,[28] Plagge, Maney and Gerhardt,[32] and others.

Modified pressure or puncture tests with cherries and the small fruits have also been reported on by Hawkins and Sando,[17] Hartman,[12, 16] Willaman, Pervier and Triebold,[39] and Verner.[41] The ability to measure definitely the firmness of the flesh or the toughness of the skin or outer covering has thus served to add considerably to the knowledge of fruit maturity, handling, shipping, and storage problems.

Tests to determine the rate at which a number of the deciduous fruits soften both before and after harvesting were begun at the California Agricultural Experiment Station in 1924. Firmness was recorded by the use of the Magness and Taylor tester. A plunger point 7/16 inch in diameter was used for plums, apricots, and apples

and a 5/16-inch point for peaches and pears. After removing the skin, tests were made on opposite sides of each of 10–20 representative fruits, and the readings averaged to constitute the firmness of the sample. A part of each lot of fruit obtained was tested for firmness immediately upon harvesting. Other portions were used for sugar and acid analyses or held for various lengths of time under different cold storage and refrigerator car temperatures.

From the beginning it was evident that not only different fruits but different varieties of the same fruit were of different texture at the time of harvesting. Moreover, it was soon apparent that Bartlett pears grown under relatively cool temperatures and high humidities were of a softer texture, compared to their color development, than fruit grown in the warmer, drier districts. The reasons for these differences are now being investigated. It would appear, however, from the work of other investigators that differences in firmness and comparative rate of softening are closely associated with differences in thickness or anatomical structure of the cell walls and upon the rapidity of certain chemical or enzymatic changes affecting their decomposition. Appleman and Conrad[(5)] report that the softening of peaches parallel the transformation of protopectin into pectin. Haller[(10)] working with apples believes this to be true with apples held in storage but that it does not account for softening on the tree or for the differences in firmness between varieties. Addoms, Nightingale and Blake[(1)] report that with the maturation of Elberta peaches the cell walls thin rapidly and show a rounding off. There is also a breaking of some of the walls resulting in a more juicy flesh. In the Shipper Cling variety these changes did not occur.

Softening of fruit following its removal from the tree is primarily dependent upon the temperature at which it is held. Softening, however, may be materially checked by the use of artificial or controlled atmospheres as has been amply demonstrated by Kidd, West and Kidd[(18)] and by Thornton.[(38)] The results on softening reported upon in this publication, however, apply only to fruit held in a normal atmosphere.

Plums.—Table 7, briefly summarizing some of the data presented previously[(2)] is sufficient to show the rapid softening of plums after changing their ground color. The pressures presented also serve to illustrate the range of softening between early and late varieties. In correlating the rate of softening with color changes, the early, soft-fleshed varieties such as Beauty, Formosa or Climax, often showing a reduction in firmness of one pound a day, must be harvested when of a straw color or when showing only slight red color

at the tip. Numerous varieties similar to Duarte and Diamond may safely be allowed to develop 50 to 75 per cent of their color, whereas President, a late, firm-fleshed variety, softening approximately only 25 per cent as rapidly as Beauty, should be allowed to develop full

TABLE 7*

SOFTENING AND COLOR CHANGES IN PLUMS

Variety	Color stage when picked	Firmness, pounds
Beauty	Green to straw tip	13.2
	†Straw to slight pink tip	9.0
	Straw to red tip	6.1
	½ to ¾ red	4.9
Burbank	Straw tip to light straw	20.7
	†Full straw to yellow	15.4
	Yellow to ¼ red	10 9
Climax	Green to faint straw tip	25.1
	Straw to greenish yellow	20.7
	†Greenish yellow to red tip	15.5
	¼ to ¾ red	8.9
Duarte	Green to slight red	15.0
	†½ to ⅔ light red	12.0
	Light to medium dark red	10.4
Diamond	Green, slight purple	16.5
	†½ to ⅔ purple	12.5
	¾ to full purple	9.2
Giant	Slight pink	18.7
	†½ pink	16.3
	⅔ to full red	10.7
President	⅓ blue	13.7
	½ blue	12.2
	¾ blue	10.5
	†Full blue	10.0
Santa Rosa	Greenish yellow to pink tip	19.0
	†¼ to ¾ color	15.4
	¾ to full light red	9.1
Wickson	Green to straw tip	21.2
	†Straw tip to straw	13.6
	Light yellow	8.2

* Averages from table 2, Agr. Exp. Sta. Bul. 428.
† Prime harvesting condition for eastern shipment.

color before harvesting. While, therefore, plums are harvested commercially when at a certain color stage, the amount of color which they are allowed to develop is based upon the texture of the variety and the rapidity of softening.

Under air temperatures at the time of harvesting, stone fruits soften rapidly after picking. Plums, apricots, and peaches, testing from 12 to 20 pounds when picked, will, when in prime eating condition, 6 to 10 days later, give a pressure test of only 1 to 2 pounds. Placed under a temperature of 32° F such fruits may be held for several weeks with little softening. Relative softening of ten varieties of plums held at 43°, and at 52°, for periods of 6 and 12 days is given in table 8. At the end of 12 days, fruit held at 43° is nearly as firm,

TABLE 8*

THE FIRMNESS OF PLUMS AFTER SIX AND TWELVE DAYS UNDER REFRIGERATOR CAR TEMPERATURES

Variety	Number of samples	Firmness when picked	Firmness after 6 days		Firmness after 12 days	
			At 43° F	At 52° F	At 43° F	At 52° F
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
Beauty	4	13 5	8.7	4.1	4.5	2.8
Formosa	3	9.9	9.7	6 3	8.0	3.4
Climax	3	11 4	10.2	4.7	4.7	2.9
Santa Rosa	2	16.0	12.4	4.2	4.6	2.9
Burbank	4	14.3	8.5	4.7	3.9	2.9
Wickson	4	18.2	12.9	11.4	7.9	6.4
Duarte	4	16.2	10.1	7.2	5.0	3.6
Diamond	4	15.7	10.6	7.6	6.4	4.2
Giant	3	19.0	16.0	6.8	5.9	3.5
President	4	11.6	11.0	8.3	6.4	4.0
Average		14.5	11.0	6.5	5.7	3.6

* Summarized from data in table 3, Agr. Exp. Sta. Bul. 428.

and in fact with the early varieties slightly firmer, than comparable samples held only 6 days at 52° F. At the higher temperature plums also soften more rapidly during the first 6 days than during the second 6 days. This is most noticeable with the early varieties. Under a temperature of 43° ripening is more uniform but frequently a smaller change in firmness takes place during the first half of the holding period than during the latter half.

Peaches.—Although somewhat firmer than plums, peaches ripen similarly to them in that they soften rapidly after they begin to show change of color. In this respect, however, variety differences must again be considered. A comparison of the Elberta with the J. H. Hale, table 9, shows that the latter is much firmer at comparable color stages than the former. It is consequently allowed to attain materially more color before harvesting. The greater color and firmness of J. H. Hale over the Early Crawford and Slappey varieties

TABLE 9

SOFTENING AND COLOR CHANGES IN PEACHES PREVIOUS TO AND DURING HARVEST

Variety	Date harvested	Color stage	Firmness, pounds
Early Crawford	July 1, 1926	Light green, faint blush	18.7
	July 7, 1926	Greenish white, slight blush	14.2
	July 12, 1926	Yellow, slight to ⅓ red	10.0
Early Crawford	July 14, 1925	Yellowish green, slight blush	13.8
	July 14, 1925	Light yellow, ¼ red	11.5
	July 20, 1925	Medium to full yellow, ¼ to ½ red	11 1
Early Crawford	July 14, 1925	Yellowish green, slight blush	20.0
	July 20, 1925	Greenish yellow, ¼ red	16.4
	July 25, 1925	Medium to full yellow, ¼ to ½ red	12.5
Elberta	July 13, 1926	Light green to greenish yellow	16.5
	July 23, 1926	Greenish yellow, slight blush	12.7
	July 23, 1926	Greenish yellow, ⅓ red	7.7
	July 27, 1926	Yellow, ⅓ red	5.7
Elberta	July 13, 1926	Green, slight blush	16.6
	July 23, 1926	Greenish yellow, slight blush	12 3
	July 27, 1926	Light to full yellow, ⅓ red	6.2
Elberta	July 20, 1925	Yellowish green, slight blush	17.6
	July 30, 1925	Cream to light yellow, slight blush	12.4
	August 6, 1925	Full yellow, ⅓ to ½ red	3.7
J. H. Hale	July 28, 1930	Greenish yellow to light yellow	16.0
	July 28, 1930	Full yellow, with blush	9.5
	July 28, 1930	Orange red	6.7
Levy	September 2, 1925	Light green, slight blush	13 6
	September 15, 1925	Greenish yellow, slight blush	7.9
	September 15, 1925	Golden yellow, ⅓ to ⅔ red	6.7
Phillips Cling	August 17, 1925	Greenish yellow to yellow	12 0
	August 25, 1925	Yellow, ¼ to ½ red	8.8
	September 2, 1925	Golden yellow, ¼ to ¾ red	8.4
Triumph	June 4, 1926	Yellowish green, ⅓ to ½ red	13.3
	June 9, 1926	Greenish yellow, ½ red	13.5
	June 16, 1926	Yellow, ¾ to full dark red	4 7
Triumph	June 5, 1925	Greenish white, slight red	14.8
	June 11, 1925	Yellowish green, ½ red	10.3
Tuskena	July 6, 1926	Greenish white, slight blush	13.5
	July 7, 1926	Yellow, ⅓ blush	11.9
	July 12, 1926	Yellow, ½ red	10.2
	July 16, 1926	Yellow, ¾ to full red	8.8
Tuskena	July 13, 1926	Full yellow, ½ red	10.5
	July 17, 1926	Full yellow, ½ to ¾ red	9.6
	July 20, 1926	Full yellow, ½ to ¾ red	10.5
	July 27, 1926	Full yellowish red	7.5

are mentioned by Blake.[6] Medium and late clingstone varieties are characterized by a firm to slightly tough flesh and a high degree of color before being considered in prime condition for harvesting. Average picking pressures for these varieties approximate 8 to 9 pounds as compared with 12 to 14 pounds for Elberta. The latter variety may often show a reduction in firmness from ½ to 1 pound per day while ripening. Softening as well as coloring of some of the early peach varieties is very uneven, the fruit being perhaps in prime eating condition on the exposed side while still hard and of green color on the opposite side.

TABLE 10

THE FIRMNESS OF PEACHES AFTER SIX AND TWELVE DAYS UNDER REFRIGERATOR CAR TEMPERATURES

Variety	Number of samples	Firmness when picked	Firmness after 6 days		Firmness after 12 days	
			At 43° F	At 52° F	At 43° F	At 52° F
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
Early Crawford	3	14.3	12.4	9.5	5.4	2.2
Elberta	3	12 3	10.8	5.3	4.9	2.5
Elberta	3	11.6	9.4	5 3	6.1	2.5
Triumph	3	12.4	10 4	9.2	7.3	2.2
Tuskena	3	10.3	10.2	9.6	8.7	3.7
Average, 15 samples		12.2	10.6	7.8	6 5	2.6

Table 10 shows that following harvest and while stored under refrigerator car temperatures, peaches soften rather rapidly, the relative amounts of softening being similar to that of plums. Held at 43° F for 12 days the fruit softens less during the first half of the period than during the second half. Under a temperature of 52° there is little difference in this respect.

Apricots.—Limited work with apricots has given results similar to those secured with plums and peaches. Early varieties tend to soften more rapidly and have a lighter yellow color when ripe than most later varieties. Royal, the principal midseason shipping variety is, when grown under valley conditions and fully ripe, of an attractive golden yellow color. During the last week or ten days on the tree it may soften as much as 1 pound per day. Tilton, ripening some two to three weeks later, is characterized by a deep yellow to orange yellow color, often with a considerable blush. The firmness of the flesh and the slower rate of softening makes it possible to allow much more color development on this variety than would be possible in most instances with Royal. Table 11 illustrates both the rate of

softening of the fruit and the correlation of color and firmness when samples of different color development are harvested the same day.

Tests on the softening of apricots after harvesting have been limited to the Blenheim variety grown in the Santa Clara Valley.

TABLE 11

SOFTENING AND COLOR CHANGES IN APRICOTS DURING HARVEST, 1930

Orchard No.	Variety	Date picked	Color stage	Firmness, pounds
1	Derby Royal	June 2	Yellowish green	16.6
		June 7	Greenish yellow	13.8
1	Early Royal	June 3	Light green to yellowish green	18.5
		June 3	Yellowish green to greenish yellow	15.8
1	Royal	June 2	Yellowish green	19.6
		June 7	Yellow	13.4
2	Royal	June 17	Yellowish green	19.0
		June 17	Yellow to orange	10.2
3	Royal	June 18	Yellowish green	14.5
		June 23	Greenish yellow	10 0
		June 23	Greenish yellow to yellow	7.1
		June 23	Yellow to orange	4.1
4	Royal	June 20	Yellowish green	17.4
		June 20	Greenish yellow	13.2
		June 20	Yellow to orange	5.8

TABLE 12

THE FIRMNESS OF APRICOTS AFTER TWELVE DAYS UNDER REFRIGERATOR CAR TEMPERATURES; 1930

Date picked	Firmness when picked	Color when picked	Firmness after 12 days at 43° F	Color after 12 days at 43° F	Firmness after 12 days at 52° F	Color after 12 days at 52° F
	pounds		*pounds*		*pounds*	
June 23	23.4	Straw	3.6	Greenish yellow	3.0	Greenish yellow
June 23	7.4	Light yellow	1.5	Light to full yellow	1.7	Light to full yellow
June 23	4.9	Full yellow	1.2	Full yellow	1.1	Full yellow

Fruit picked at a straw color stage with a firmness of 23.4 pounds softened in 12 days at 43° F, to 3.6 pounds, and to 3 pounds when held at 52°. The fruit was eating ripe in both instances 2 days later. Additional samples picked when light yellow and at a pressure of 7.4 pounds were fully ripe after 12 days. Fruit averaging 4.9 pounds when picked shows similar ripening but was slightly softer

and of higher color than that picked at a pressure of 7.4 pounds (table 12). It would appear, therefore, that apricots soften very rapidly at temperatures as low as 43° F. Pressure tests were not recorded on fruit held at 32° for four to eight weeks. In observing such lots, however, it was noted that unless the fruit was well advanced in its maturity when stored, the flesh became tough and leathery rather than soft.

Pears.—Extensive tests on the softening of Bartlett pears were made during the seasons of 1925–1928 and the results given in detail in two previous publications.[3, 27] Under moderate temperature and humidity conditions with a continuous supply of available soil moisture, the firmness of Bartlett pears, between the earliest and latest pickings, was found to decrease from 2 to 3 pounds every 10 days. This softening not only occurs during and immediately preceding the harvest period, but the data in table 13, representing the results from four individual orchards in different districts, shows that it can be measured from the time the fruit is one-third to one-half grown, and that the rate of softening in the earlier stages of development does not appear to be materially different from that six to eight weeks later (table 13 and fig. 1).

Softening as indicated by the fruit from these orchards may be considered as typical for the Bartlett variety. Climatic influences and the relative length of time during the development of the fruit that the trees may suffer from a shortage of available soil moisture are, however, important influencing factors upon both softening and color development. Fruit grown in the hotter districts with a low humidity and under a deficiency of soil moisture may attain a high color with relatively little softening. In the cooler districts, subject to coastal influences, changes in texture of the flesh are much more marked than changes in color. Aside from soil moisture and climatic influences Bartlett pears produced on trees growing on the Japanese (*Pyrus serotina*) rootstock were found to have a somewhat firmer flesh than those grown on French (*P. communis*) roots.[4]

Detailed storage tests of Bartlett pears have shown that fruit held at 31° F exhibited a reduction in firmness of only 1 to 2 pounds in three months, while at 36° the softening was found to be from two to three times as rapid. Held at 43° the fruit showed only slight softening (a reduction in firmness of 1 to 2 pounds) for the first 12 to 18 days. Following this initial period, however, softening was relatively rapid, pressure test readings decreasing approximately 2 pounds a day. Under a temperature of 52° F samples having a firmness of 18 to 20 pounds when picked, tested only 5 pounds after

12 days, or softening over 50 per cent more rapidly than comparable samples held at 43° F. Initial softening was apparent within 6 days after harvesting. Differences in ripening between 43° and 52° F are due primarily to the more rapid initial softening at the higher temperature.[27]

TABLE 13

COLOR CHANGES AND SOFTENING OF BARTLETT PEARS PRECEDING AND DURING THE PERIOD OF HARVESTING; 1927

Location of orchard	Date picked	Diameter of fruit, inches	Color*	Firmness, pounds	Average amount of softening each 10 days, pounds
Santa Clara Valley	June 15	¾	1	28–30+	
	July 1	1½	1	29.0	
	July 13	1½–2	1½	27.4	
	July 20	2–2¼	1½	23.4	
	July 27	2½	1½–2	21.3	
	†August 4	2½	2	19.4	
	August 10	2½	2–2½	18.0	
	August 23	2½	2½	16.0	2.0
Sacramento River district	June 20	1¼–1½	1	28–30+	
	June 29	2–2¼	1–1½	26.9	
	†July 6	2¼	1–1½	23.5	
	July 19	2¼–2½	1½–2	22.6	
	July 26	2½	2	19.5	
	August 6	2½	2½	17.3	2.7
Newcastle	July 1	1¾–2	1–1½	29.2	
	July 13	1¾–2	1–1½	28.6	
	†July 20	2–2¼	1½–2	26.9	
	July 27	2½–2¾	1½–2	21.5	
	August 3	2½	2–2½	21.0	
	August 17	2½	2½	17.5	
	August 25	3	3	15.2	2.5
Placerville	July 14	2	1	30.0+	
	July 25	2	1	29.2	
	†August 3	2¼	2	24.8	
	August 10	2½–2¾	2	20.5	
	August 17	2¾	2½	17.6	
	August 24	2½	2½	17.0	
	September 1	2¾	3	15.8	2.9

* The colors corresponding to the numerical values are: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow.

† Dates on which the first fruit was picked commercially.

Maturity studies extending over three seasons on late summer, fall and winter varieties show that these have a flesh only one-half to two-thirds as firm as that of the Bartlett. Changes in firmness immediately preceding and during the period of harvesting are correspondingly less marked, except for short periods in occasional

instances. Beurre Hardy, Comice, Anjou, Beurre Bosc, Winter Nelis, and Easter Beurre, have occasionally been noted to have softened between early and late pickings as rapidly as the Bartlett; while in other instances the fruit may fail to show any softening over a 10-day period.

Between the above extremes, found in a few cases, most samples softened from ½ to 1½ pounds in 10 days or approximately half as much as most usually occurs in the Bartlett. Table 14 illustrates a few typical cases of softening of the most important commercial varieties. In general the rate of softening of the different varieties does not vary greatly. On the average, Beurre Hardy and Winter Nelis failed to soften quite so rapidly as Comice, Anjou, Beurre Bosc and Easter Beurre. Winter Nelis was rather variable in softening, frequently showing little or no change in firmness over a period of two to three weeks.

TABLE 14

THE SOFTENING OF FALL AND WINTER PEARS PRECEDING AND DURING THE PERIOD OF HARVESTING

Variety	Orchard No.	Date picked	Color*	Firmness, pounds	Average amount of softening each 10 days, pounds
Beurre Hardy	1	July 24, 1928	1	12.3	
		August 11, 1928	2	10.8	
		August 30, 1928	2½-3	8.6	
					1.0
	2	July 24, 1928	1	11.4	
		August 11, 1928	2	10 9	
		August 20, 1928	2	10.5	
					0.3
	1	August 9, 1929	2	11.8	
		August 21, 1929	2-2½	10.8	
		September 1, 1929	2-2½	9.8	
					0.9
	2	August 9, 1929	2½	11.3	
		August 21, 1929	2½	10 5	
					0.6
	3	August 9, 1929	2	10.8	
		August 22, 1929	2½	9.7	
					0.9
	4	August 9, 1929	2 -2½	10.7	
		August 22, 1929	2½-3	9.8	
					0.7
Comice	1	September 1, 1929	3	9.4	
		September 11, 1929	4	8.7	
					0.7
	2	August 22, 1929	2½	12.0	
		September 1, 1929	2½	10.3	
					1.7

* The colors corresponding to the numerical values are: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow.

TABLE 14 (*Continued*)

Variety	Orchard No.	Date picked	Color*	Firmness, pounds	Average amount of softening each 10 days, pounds
Comice	3	August 22, 1929	2-3	12.4	
		September 1, 1929	3	10.5	1.9
	4	August 13, 1930	2-3	10.0	
		August 22, 1930	3	10.3	
		August 30, 1930	3	9 1	0.5
	5	August 14, 1930	2-3	9.4	
		August 23, 1930	3	9.3	
		August 30, 1930	2-2½	8.6	0.5
	6	August 28, 1930	3	9.8	
		September 17, 1930	4	6.7	1.5
	7	August 19, 1930	2	10.7	
		August 30, 1930	2-3	9.6	
		September 20, 1930	2½	7.7	0.9
Anjou	1	August 9, 1929	2	15.8	
		August 22, 1929	2	13.2	2.0
	2	August 22, 1929	2	14.2	
		September 1, 1929	2	12.5	1.7
	3	August 13, 1930	2	14 3	
		August 30, 1930	2	13.0	0.8
	4	August 29, 1930	2½	12.0	
		September 20, 1930	2-2½	9.8	1 0
Beurre Bosc	1	August 20, 1928	1 -1½	15 0	
		August 30, 1928	1½-2	12.2	
		September 26, 1928	2-3	9.7	1.4
	1	August 22, 1930	2½	13.2	
		August 30, 1930	3	9.9	
		September 12, 1930	3	9.5	1.6
	2	August 22, 1930	2½-3	12 4	
		August 30, 1930	3	11.1	
		September 12, 1930	3	10.0	0.7
	3	July 30, 1930	1-2	15.5	
		August 11, 1930	3	14.5	
		August 13, 1930	3	13.9	1.1
Winter Nelis	1	September 1, 1929	....	15.5	
		September 10, 1929	....	15.6	
		September 18, 1929	....	15.3	0.1
	1	September 12, 1930	....	13.5	
		September 23, 1930	....	13.5	0.0

* The colors corresponding to the numerical values are: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow. Winter Nelis predominantly russet.

TABLE 14 (*Continued*)

Variety	Orchard No.	Date picked	Color*	Firmness, pounds	Average amount of softening each 10 days, pounds
Winter Nelis	2	September 1, 1929		16.9	
		September 10, 1929		16.6	
		September 19, 1929		15.6	0.7
	2	August 30, 1930		15.9	
		September 12, 1930		14.8	
		September 23, 1930		14 0	0 8
	3	September 19, 1929		15.5	
		September 27, 1929		15 4	0.1
	3	August 23, 1930		14.8	
		August 30, 1930		14.0	
		September 12, 1930		13 9	
		September 23, 1930		12.0	0.9
	4	September 18, 1929		14 8	
		September 28, 1929		13 7	1.1
	5	August 28, 1930		20 8	
		September 9, 1930		19.8	
		September 20, 1930		17 7	1 3
Easter Beurre	1	October 11, 1928	2	15 9	
		October 20, 1928	2	14.7	1.3
	2	September 11, 1930	1½-2	17.0	
		September 18, 1930	2	16.3	1.0
	2	August 30, 1930	2	16.0	
		September 12, 1930	2	14.7	1.0
	3	August 30, 1930	1	16.3	
		September 10, 1930	2	15.0	1.2
	4	August 30, 1930	1	18.9	
		September 12, 1930	2	15 3	
		September 22, 1930	1	11.8	3 0
	5	August 30, 1930	1	14.5	
		September 22, 1930	1	13.0	0.6

* The colors corresponding to the numerical values are: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow. Winter Nelis predominantly russet.

Although winter pears soften more slowly on the tree than the Bartlett yet when held in 32° storage for 14 to 16 weeks they show a similar amount of softening. Table 15 shows an average decrease of 2.6 pounds in firmness between September and the last of December. The effects of a 10-day delay at 50° F before storing at 32° is also shown in table 15. During this 10-day period the fruit softened

nearly as much as it did in 14 weeks at 32°. Notwithstanding this fact, the differences in firmness of fruit stored immediately at 32° and that stored after a 10-day delay, averaged only 2 pounds. A comparison of Bartlett pears held under similar conditions has shown that the fruit held for 10 days at 50° F has a pressure test reading of only 3 to 5 pounds, and is either fully ripe or very close to prime eating condition.

TABLE 15

FIRMNESS OF WINTER NELIS PEARS, DECEMBER 29, 1930, FOLLOWING IMMEDIATE STORAGE AT 32° F AND STORAGE AFTER A 10-DAY SHIPPING PERIOD AT 50° F

Locality grown	Orchard No.	Date picked	Firmness, pounds		
			When picked	Following immediate storage at 32°	Following storage at 32° after 10 days at 50°
Placer County	1	September 17	15.5	12 9	9.7
	2	September 17	15.0	11.4	9 7
Santa Clara Valley	1	September 22	14 2	11 5	8.1
	2	September 22	13.1	10.7	9.0
	3	September 22	13.5	11 1	10 3
	4	September 22	14 0	10.8	9 3
	5	September 22	12.0	10 5	9.2
Average			13.9	11.3	9.3

Apples.—The Gravenstein variety, harvested in July, ripens relatively quickly, softening on the trees being about one-half that usually occurring in Bartlett pears. The rate of softening in the different orchards and in the two districts from which fruit was secured proved to be very similar. The two orchards in the Napa district, however, where the trees may at times have suffered for lack of available water, and growing under higher temperatures and lower humidity conditions, produced a slightly firmer fruit than that from the Sebastopol orchards (table 16).

Softening of the Yellow Bellflower and Yellow Newtown is similar to that of the Gravenstein, although in general, hardly so rapid.

During the first six days after picking, pressure tests of four separate samples of Gravensteins show, as with pears, that there is little difference in the rate of softening at 43° and 52° F (table 17). From the sixth to the twelfth day, softening became more rapid and the differences in firmness at the two temperatures became more marked, being 1.8 pounds at 43° as compared with 3 pounds at 52°. This difference, although small, was consistent in a large proportion

TABLE 16

COLOR CHANGES AND FIRMNESS OF APPLES PRECEDING AND DURING THE PERIOD OF HARVESTING

Variety	Locality grown	Number of orchards averaged	Date picked	Color*	Firmness, pounds	Average amount of softening in 10 days, pounds
		3	June 11, 1926	1–2	20.7	
		3	June 18, 1926	2	18.8	
Gravenstein	Sebastopol	4	June 25, 1926	2–3	18.3	
		4	July 2, 1926	2–3	17.4	
		4	July 8, 1926	2–3	17.0	
		4	July 22, 1926	2½–3½	14.9	
						1.4
		6	July 10, 1929	2–2½	18.8	
Gravenstein	Sebastopol	7	July 19, 1929	2–2½	17.1	
		7	July 27, 1929	2–2½	17.6	
		5	August 7, 1929	3–4	16.0	
						1.0
		2	July 10, 1929	1½–2	22.2	
Gravenstein	Napa	2	July 19, 1929	1½–2	19.2	
		2	July 27, 1929	2	18 8	
		2	August 7, 1929	3–3½	17.9	
						1.4
		6	August 13, 1929	2–2½	21.4	
		6	August 21, 1929	2–2½	19.8	
Yellow Bellflower	Watsonville	6	August 31, 1929	2–3	20.1	
		6	September 10, 1929	2–3	18.1	
		3	September 18, 1929	2–3	17.2	
		2	September 27, 1929	2–3	15.7	
						1.2
		2	August 30, 1928	1	21.8	
Yellow Newtown	Watsonville	3	September 13, 1928	1½–2	20.2	
		2	September 20, 1928	1½–2	20.0	
		3	October 4, 1928	2–3	18.0	
						1.1

* The colors corresponding to the numerical values are: 1. original green; 2. light green; 3. yellowish green; 4. greenish yellow to light yellow.

TABLE 17

FIRMNESS OF GRAVENSTEIN APPLES AFTER SIX AND AFTER TWELVE DAYS UNDER REFRIGERATOR CAR TEMPERATURES; 1926

Date picked	Number of orchards averaged	Firmness when picked	Firmness after 6 days*		Firmness after 12 days	
			At 43° F	At 52° F	At 43° F	At 52° F
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
June 11	3	20.6	20.0	19.7	18.5	17 0
June 25	4	18.5	19.2	18.7	16.1	15.4
July 8	5	17.4	18.1	17.8	16.7	14.8
July 22	5	15.4	15.0	14.3	13.4	12.5
Average		17.9	18.1	17.6	16.1	14.9

* The apparent gain in firmness of two of the samples after 6 days at 43° is due to variation in sampling and perhaps also to a slight lack of turgidity of the fruit.

of the individual samples. After the 12-day period when the fruit at 43° had begun to soften appreciably the softening changes were about as rapid at 43° as they were at 52°. All samples were considered eating ripe on the twenty-first day following picking. In four out of five orchards the fruit held for 12 days at 52° had slightly higher color than that held at 43°.

Midseason pickings from five orchards, with an average pressure test of 17.8 pounds, showed a decrease of 2.8 pounds in 10 days storage at 50°, and 4.6 pounds in the same length of time at 70° F (table 18).

TABLE 18

FIRMNESS OF GRAVENSTEIN APPLES WHEN HELD FOR TEN DAYS AT 50° AND AT 70° F; 1930

Orchard No.	Date picked	Firmness when picked	Firmness after 10 days	
			At 50° F	At 70° F
		pounds	*pounds*	*pounds*
1	July 14	18 6	16.4	13.3
2	July 14	16.9	14.2	11.2
3	July 21	17.6	16.9	14.4
4	July 21	17 9	15 2	12.9
5	July 21	18.2	16 6	14.2
Average		17.8	15.0	13.2

CHANGES IN SOLUBLE SOLIDS

In extracting the fruit juices for soluble solid determinations the usual method was to run the samples of fruit through an Enterprise fruit press and then filter the juice and pulp through several folds of cheesecloth. With some of the more juicy varieties of peaches and plums, grinding was unnecessary. With other varieties of plums and also Beurre Bosc and Winter Nelis pears, considerable difficulty was encountered in extraction owing to the fact that when pressed the individual fruit cells would break apart rather than rupture, resulting in a very thick sap or pulp butter. Centrifuging the material was of some value but not always satisfactory. In order to secure readings with relatively small quantities of juice and to have a relatively large reading scale, two 6-inch Balling hydrometers were used, one reading from 0 to 10 per cent and one from 10 to 20 per cent. The usual corrections were made for variations in temperature.

Plums.—Soluble solids of plums immediately preceding and during the time of harvest were found to run from 8.1 to 16.3 per cent

(table 19). Samples of fruit of different color stages picked concurrently show that soluble solids, as well as sugars and acid, are closely associated with color development. Previous to and during the early stages of color development, soluble solids increase rather slowly while, after the characteristic overcolor of the fruit begins to appear, the increase is rapid. The quantity of soluble solids is approximately twice that of the total sugars and the percentage increase between that in very early-picked fruit and that in fruit of full color has been found to be as much as 15 to 25 per cent. After harvesting and until ripe, the soluble solids remain practically constant.[2]

Peaches.—Hydrometer readings of the juice of six varieties of peaches listed in table 20 show the soluble solids to fluctuate within a relatively narrow range around 12.0 per cent, with a minimum of 10.5 and a maximum of 14.5 per cent. In general the soluble solids tend to increase with the coloring and the softening of the fruit although the changes during this period are not always consistent and the differences are much less marked than with plums. Thompson and Whittier[37] have shown that soluble solids increase over the entire development period of the peach.

In the majority of samples there appears to be a slight gain in the soluble solids after picking. This is in agreement with the data of Culpepper and Caldwell[7] and is, as they state, doubtless due in large part at least to a loss in moisture from the fruit during ripening in storage. Similar gains were also noted in total sugars, which would tend to substantiate this explanation.

Pears.—Hydrometer readings on the juice of Bartlett pears, table 21, show a gradual increase in soluble solids with increase in color and with softening of the fruit, this increase continuing after harvesting until the fruit is ripe. Loss of moisture may account for a small amount of this increase but as all fruits were individually wrapped the greater part of the gain in solids and sugars is ascribed to the hydrolysis of starch.

Apples.—No determinations were made with apples to ascertain the changes in soluble solids immediately preceding and during harvest. Thompson and Whittier,[37] however, found late apples to increase in soluble solids throughout their development period.

CHANGES IN SUGAR CONTENT

For the purpose of analysis, small longitudinal slices, extending from the surface to the core and totaling 50 grams in weight, were cut from 10–20 fruits and placed in a 250 cc Erlenmeyer flask. The sample of fresh fruit was then covered with 150 cc of 95 per cent alcohol and brought to a brisk boil. For convenience the material was stored at this point. Further extraction of the fruit tissue, using approximately 100 cc of 95 per cent alcohol was carried out in Soxhlet thimbles, extracting for 8 hours. The alcohol used for extracting, together with that in which the sample was originally put up, was transferred into a 500 cc volumetric flask and made up to volume.

Aliquots of from 75 to 100 cc were placed in 300 cc Erlenmeyer flasks and the solution evaporated to dryness under a vacuum on a water bath at not over 60° C. The residue was taken up in water, cleared with neutral lead acetate and centrifuged. The clear liquid was then deleaded with potassium oxalate and the sugars determined by the Shaffer-Hartmann modification of the Munson-Walker method.[33]

Plums.—Representative data giving the development of sugars and their association with color development are shown in table 19. All ten varieties previously reported showed a marked increase in sugar content as the fruit ripened on the tree, the increase being 10 to 15 per cent for each commercial picking stage. The sugar content increases at a relatively uniform rate from the early picking stages until the fruit is of full color. As previously mentioned, soluble solids increase slowly during the early maturity stages of the fruit; hence, the ratio between soluble solids and sugar is not constant. In well colored fruit the percentages of sugars are usually slightly more than half the hydrometer readings, while in earlier picked fruit the sugars may be slightly less than half that of the soluble solids. Reducing sugars are relatively stable in quantity throughout the ripening period but usually increase slightly. Sucrose has been found to be approximately twice as great in late-picked samples as in those picked early.[2] Different varieties vary in the proportion of sucrose to total sugars which they contain. After harvesting and until fully ripe, there is little change in either the amount or relative proportions of the sucrose and reducing sugars.

Peaches.—With slight fluctuations when picked at intermediate stages of ripeness, total sugars in peaches between the earliest and latest dates show a relative increase in sugar of from 10 to 17 per cent.

Table 20 gives the actual increase at different stages of color and firmness of the fruit. The later ripening varieties of cling peaches contain a somewhat higher percentage of sugars than earlier varieties. Reducing sugars were found to show either a slight increase or decrease between individual pickings with no very definite tendency in either direction. Culpepper and Caldwell[7] report a general decrease, followed in some samples by a slight increase when the fruit becomes very soft. This increase in the riper fruit is noted in some of the samples. In all samples the differences between the reducing sugars and total sugar become larger as the fruit ripens. There is therefore a distinct gain in the sucrose content of the fruit. Culpepper and Caldwell report considerable variation in the sugar content in different seasons.

Following harvest, the quantity of sugars averages slightly higher than when picked, the apparent gain in total sugars again being attributed primarily to water loss.

Apricots.—Analyses were run on only a few samples of apricots and are given in table 22. The data show a marked and consistent increase in both reducing and total sugars as the fruit matures. Increase in sucrose is particularly marked between the first and second color stages of early-picked fruit. Only slight changes in any of the sugars were apparent after the fruit had been held at 50° for 10 days at which time the more mature samples were near prime eating condition.

Pears.—Bartlett pears make a material gain in sugars both as they ripen on the tree and after picking (table 21). Early-picked samples fail to develop as much sugar when ripe as do those picked late. The actual increase after picking in the early-picked fruit may, however, be as great as, if not greater than, in that picked later. As reported by Magness[21] increase in sugars, particularly early in the season, is primarily due to the increase in reducing sugars, which according to Thompson and Whittier,[36, 37] are largely levulose. At the time of commercial harvesting, sucrose is present in small amounts. As the fruit ripens at 70° F following harvest, the increase in sugar present is the result of an increase in both sucrose and reducing sugars. The amount of sucrose tends to be somewhat greater in fruit well matured before harvesting although some of the earlier samples show as much as those picked later.

Apples.—Data presented in table 23 on sugar changes in Gravenstein apples are in conformity with the general findings of Magness and Diehl,[22] St. John and Morris,[35] of Plagge, Maney and Gerhardt[32] and of other earlier investigators. With the decrease of

TABLE 19

SOLUBLE SOLIDS, SUGARS, AND ACID CONTENT OF PLUMS IN RELATION TO COLOR DEVELOPMENT; 1926

Variety	Date picked	Color stage	Soluble solids	Reducing sugars	Total sugar	Malic acid in juice	Malic acid in fruit
			per cent Balling	*per cent*	*per cent*	*per cent*	*per cent*
Beauty	June 2	Straw to slight pink tip	11 3	3.80	4.34	1.43	....
		Pink tip to ⅓ red	11.8	5.80	6.20	1.02	....
	June 12	Greenish yellow to red tip	11.5	6 01	....	1.10	2.03
		⅔ to full red	13.4	6.34	....	0.94	2.29
Burbank	June 25	Straw to yellow	14.5	3.87	9.12	1.55	1.79
		Straw with red tip	14.2	3.81	9.17	1.36	1.73
	July 6	Yellow, slight red	16.3	3.49	10.37	1.04	1.46
		Yellow, ½ red	16.2	3.70	11.06	0.92	1.41
	July 9	Yellow	14.1	2.94	8.46	1.05	1.68
		Yellow, ¼ red	14.0	3.07	9.27	1.04	1.64
Climax	June 13	Greenish yellow to full straw	10.1	4.58	....	1.84	2.12
		Greenish yellow, red tip	11.5	4.84	....	1 75	2.42
	June 16	Green with faint straw tip	10.2	4.76	....	2.37	2.31
		Green with slight red tip	11.0	5.06	....	1.81	2.46
Diamond	July 7	Green to slight purple	10.8	4.32	5.62	....	2.60
		½ to ⅔ purple	12.7	4.35	7.09	2.51	2.50
	July 7	Green to ¼ purple	12.5	....	5.10	....	....
		⅔ purple	14.2	....	6.20	....	....
Duarte	July 3	Yellowish green, slight dull red	12.4	4.26	6.53	1.62	1.45
		½ to ⅔ light red	13.4	3.94	7.26	0.80	1.31
	July 9	Greenish yellow, slight red	11.5	4.32	6.61	1.35	1.61
		½ to ⅔ red	14.6	4.12	8.10	0.99	1.48
Formosa	June 3	Green to light straw	8.1	3.54	4 30	0.94	....
		Straw to faint pink tip	10.3	4.50	5.70	0.57	....
Santa Rosa	June 11	Pink tip	13.6	5.52	6.70	1.48	1.83
		⅓ to ½ light red	14.4	5.30	6.96	1.41	1.95
	June 11	Greenish yellow, red tip	11.7	5.15	6.24	2.19	2.85
		¼ to ¾ red	13.1	5.27	6.56	2.07	2.77
	June 13	Greenish yellow, slight red	11.1	4.50	....	2.34	2.74
		¼ to ½ red	12.1	4.45	....	2.25	2.74

TABLE 20

MATURITY CHANGES IN PEACHES DURING THE PERIOD OF HARVESTING

Orchard No.	Date picked	Color stage	Firmness	Soluble solids		Reducing sugars		Total sugar		Acid as malic	
				When picked	When ripe	When picked	When ripe	When picked	When ripe	When picked	When ripe
			pounds	*per cent Balling*	*per cent Balling*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
		Early Crawford									
1	July 14, 1925	Yellowish green, slight blush	20.0	12.2	12.1	2.40	2.75	8.06	8.97	....	0.84
1	July 20, 1925	Greenish yellow, ¼ red	16.4	12.3	12.6	2.45	2.63	9.15	9.24	0.70	0.69
1	August 6, 1925	Full yellow, ¼ to ½ red	12.5	12.5	12.7	2.39	2.57	9.24	9.36	0.71	0.72
2	July 14, 1925	Yellowish green	13.8	11.5	11.9	2.43	2.46	8.12	8.36	0.96	0.97
2	July 14, 1925	Light yellow, ¼ red	11.5	12.5	12.2	2.64	2.73	8.48	8.57	0.82	0.65
2	July 20, 1925	Full yellow, ¼ to ½ red	11.1	12.6	14.4	2.49	2.78	9.35	10.08	0.59	0.61
3	July 1, 1926	Light green	18.7	13.5	14.0	2.50	3.00	7.06	8.00	0.70	0.74
3	July 7, 1926	Greenish yellow, slight blush	14.2	14.4	13.7	2.42	2.34	8.66	8.10	0.83	0.63
3	July 12, 1926	Yellow, slight to ⅓ red	10.0	14.5	14.4	2.06	2.56	9.34	10.08	0.65	0.62
		Average	......	*12.9*	*13.1*	*2.42*	*2.64*	*8.60*	*8.97*	*0.74*	*0.72*
		Elberta									
1	July 20, 1925	Yellowish green	17.6	11.7	12.7	2.73	3.00	8.47	8.47	0.63	0.79
1	July 30, 1925	Greenish yellow to light yellow	12.4	12.1	12.8	2.72	2.94	8.30	9.09	0.62	0.53
1	August 6, 1925	Full yellow, ⅓ to ½ red	3.7	13.1	13.0	2.94	2.94	9.66	10.21	0.49	0.44
2	July 23, 1925	Greenish yellow	17.8	12.0	12.3	3.00	3.04	7.56	8.15	0.66	0.63
2	July 30, 1925	Greenish yellow to light yellow	13.2	11.3	11.0	3.05	3.04	7.86	8.18	......	0.53
3	July 13, 1925	Light green to greenish yellow	16.5	12.6	13.6	2.96	3.10	7.86	......	0.56	0.50
3	July 23, 1926	Greenish yellow, ¼ red	12.7	11.6	14.4	2.60	2.56	7.02	7.30	0.37	0.34
3	July 23, 1926	Greenish yellow to yellow, ⅓ red	7.7	11.6	11.7	2.56	2.80	7.74	8.50	0.33	0.35
3	July 27, 1926	Greenish yellow to yellow, ⅓ red	5.7	11.6	13.0	2.68	2.56	8.86	9.98	0.34	0.32
4	July 13, 1926	Green	16.6	12.7	14.3	3.10	3.26	7.86	8.66	0.52	......
4	July 23, 1926	Greenish yellow	12.3	12.1	12.7	3.10	3.40	8.50	8.10	0.41	......
4	July 23, 1926	Greenish yellow to yellow, ¼ red	6.1	12.0	12.6	3.26	3.26	8.26	8.66	0.36	......
4	July 27, 1926	Yellow, ⅓ red	6.2	13.6	13.2	3.10	3.48	9.20	9.26	0.40	......
		Average	......	*12.1*	*12.8*	*2.91*	*3.03*	*8.24*	*8.71*	*0.47*	*0.49*

TABLE 20—*Continued*

Orchard No.	Date picked	Color stage	Firmness	Soluble solids		Reducing sugars		Total sugar		Acid as malic	
				When picked	When ripe	When picked	When ripe	When picked	When ripe	When picked	When ripe
			pounds	*per cent Balling*	*per cent Balling*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
		Triumph									
1	June 5, 1925	Greenish white	14.8	11.3	10.5	1.54	1.58	6.98	7.91	0.84	0.74
1	June 11, 1925	Yellowish green, ½ red	10.3	12.0	10.7	1 55	1.76	8 33	8.12	0.96	0.71
2	June 4, 1926	Yellowish green, ⅓ red	13.3	11.9	11.6	2.20	2.00	7.90	7.40	0.47	0.33
2	June 9, 1926	Greenish yellow, ⅔ red	13.8	11.3	10 7	1.70	1.70	7.64	6.40	0.36	0 28
2	June 16, 1926	Yellow, ¾ to full red	4.7	11 9	11.2	1.60	1.82	8 90	8 66	0 36	0.30
		Average	......	*11.7*	*10.9*	*1.72*	*1.77*	*7.95*	*7.30*	*0.60*	*0.47*
		Tuskena									
1	July 3, 1925	Greenish yellow, slightly red	20.3	11.4	12.6	2.43	2 85	7.32	8 00	0.96	......
1	July 9, 1925	Greenish yellow to full yellow, ¼ red	12.9	11.7	12.7	2.18	2.43	7.81	8.30	1.00	0.96
1	July 13, 1925	Full yellow, ½ red	10.5	11.6	12.4	2.12	2.31	7.93	7.90	0.98	0.90
1	July 17, 1925	Full yellow, ½ to ¾ red	9.6	12 3	12.7	2.43	2 52	8 87	8.82	0.92	0 90
2	July 17, 1925	Greenish yellow, slight to ¼ red	13.5	11.9	11.9	2.24	2.51	7.47	7.63	1.08	0.97
2	July 20, 1925	Greenish yellow to full yellow, ½ to ¾ red	10.5	12.0	12.3	2.29	2.35	8.20	8.18	0.78	0.83
2	July 27, 1925	Yellowish red	7.5	12.0	13 2	2.17	2.04	8.08	8.12	0.88	0.87
3	July 6, 1926	Greenish yellow, slight red	13.5	12.9	13.2	2.24	2.34	8 00	8.04	1.00	0.80
3	July 6, 1926	Yellow, ⅓ red	11.9	13.7	12.8	2 10	2 10	8.74	8 40	0.76	0.66
3	July 12, 1926	Yellow, ½ dark red	10.2	13.0	13.6	2.18	2.24	9 90	9 46	0.60	0.62
3	July 16, 1926	Yellowish red	8.8	13 2	14 0	2.24	2.18	9.40	9 32	0.47	0.44
		Average	......	*12.3*	*12.9*	*2 24*	*2.35*	*8.34*	*8.38*	*0.86*	*0.79*
		Levy									
1	September 2, 1925	Light green	13.6	10.5	13 2	2 39	2.49	9.18	9.73	0.69	0.49
1	September 15, 1925	Greenish yellow, ¼ red	7.9	10.8	11.2	2.31	2.52	9.54	10.22	0 68	0.39
1	September 15, 1925	Golden yellow, ½ red	6.7	12.5	14 2	2.43	2.76	10.40	11.28	0.39	0.36
		Phillips Cling									
1	August 17, 1925	Greenish yellow to yellow	12.0	11.5	12.1	2 15	2 15	8.39	8.56	0.77	0.70
1	August 25, 1925	Yellow, ¼ to ½ red	8.8	12.4	12.7	2.40	2.52	9.82	10.10	0.63	0.56
1	September 2, 1925	Golden yellow, ¼ to ¾ red	8.4	12.6	13.1	2.40	2.63	10.22	10.73	0.57	0.45

TABLE 21

MATURITY CHANGES IN BARTLETT PEARS DURING THE PERIOD OF HARVEST

Locality	Orchard	Date picked	Color	Firmness	Soluble solids, When picked	Soluble solids, When ripe	Reducing sugars, When picked	Reducing sugars, When ripe	Total sugar, When picked	Total sugar, When ripe	Acid, as malic, When picked	Acid, as malic, When ripe
				pounds	*per cent Balling*	*per cent Balling*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Sacramento River district	1	June 24, 1925	1	24.5	....	12.6	5.32	6.20	5.53	7.00	0.52	0.39
		July 6, 1925	1	22.0	11.6	12.8	5.31	6.31	5.76	7.38	0.49	0.51
		July 16, 1925	2	19.9	11.7	12.7	6.08	6.81	6.69	7.81	0.48	0.45
		July 25, 1925	2	19.2	12.3	12.7	6.02	7.81	7.81	8.11	0.40	0.44
		August 4, 1925	2	19.3	14.7	15.5	7.01	7.42	8.22	9.64	0.51	0.45
	2	June 29, 1925	1	22.6	11.7	13.0	5.38	6.50	....	7.11	0.55	0.46
		July 10, 1925	1½	21.3	12.0	14.0	5.83	6.93	6.17	7.84	0.51	0.46
		July 20, 1925	2	19.9	13.9	14.9	7.72	7.51	8.33	8.45	0.43	0.47
		July 31, 1925	2	18.9	14.8	15.6	7.72	8.18	8.07	9.42	0.41	0.47
	3	June 8, 1926	1	26.5	11.9	13.8	4.84	6.90	4.90	7.30	0.16	0.16
		June 14, 1926	1½–2	23.9	12.0	12.4	5.14	6.40	5.44	6.90	0.12	0.13
		June 21, 1926	1½–2	21.6	12.4	13.4	5.90	5.26	6.20	7.60	0.16	0.21
		June 28, 1926	1½–2	19.7	12.1	13.3	5.66	5.66	5.94	6.30	0.17	0.19
		July 7, 1926	2–2½	19.2	12.9	13.6	5.08	7.02	5.34	7.86	0.17	0.18
		July 20, 1926	2–2½	18.9	13.6	14.8	5.94	7.38	6.62	8.70	0.16	0.17
Average					*12.7*	*13.7*	*5.93*	*6.82*	*6.50*	*7.83*	*0.35*	*0.34*
Placer County	4	July 3, 1925	1	24.3	13.0	13.4	5.65	6.96	6.74	7.93	0.62	0.63
		July 13, 1925	1–2	20.7	12.6	14.3	6.01	6.93	6.50	8.14	0.54	0.55
		July 23, 1925	2	21.2	12.6	13.9	6.05	6.93	6.81	8.21	0.76	0.50
		August 3, 1925	2	21.3	13.6	14.3	6.01	7.63	6.56	9.43	0.71	0.61
	5	July 2, 1925	1	23.6	13.0	14.1	5.77	7.26	6.00	8.51	0.66	0.74
		July 13, 1925	1–2	19.2	12.4	13.9	6.01	7.11	6.50	8.60	0.70	0.69
		July 23, 1925	2	19.2	13.0	14.5	6.32	7.20	6.81	8.42	0.71	0.68
		August 3, 1925	3	18.8	13.4	14.5	6.63	6.63	7.32	9.49	0.75	0.71
		August 12, 1925	3	17.1	13.5	14.9	6.25	7.11	7.23	9.50	0.79	0.62
	6	June 25, 1926	2	23.1	14.7	15.9	6.46	7.86	6.54	9.00	0.20	0.36
		July 2, 1926	2	19.9	13.6	14.4	5.90	7.94	6.26	8.70	0.21	0.27
		July 9, 1926	1½–2	18.3	14.7	15.0	6.54	7.80	6.86	8.86	0.28	0.28
		July 16, 1926	2–2½	17.5	15.1	16.0	7.86	8.34	8.40	9.40	0.30	0.24
		July 23, 1926	2½	17.2	15.6	17.2	6.86	8.40	7.20	9.58	0.34	0.19
		July 30, 1926	2½–3	16.0	16.3	16.3	6.92	8.58	7.52	9.94	0.30	0.24
Average					*13.8*	*14.8*	*6.35*	*7.51*	*6.88*	*8.91*	*0.52*	*0.49*

starch accompanying maturity there is a rather marked and consistent increase in sugars, the increase being due to greater amounts of both sucrose and reducing sugar. With the early season of ripening of the Gravenstein these changes continue with considerable rapidity during a 10-day holding period at 50° and at 70° F. Starch almost disappears at the higher temperature while the sugars show an average relative increase of approximately 20 per cent.

TABLE 22

SUGAR AND ACID CHANGES IN APRICOTS DURING THE PERIOD OF HARVEST; 1930

Orchard No.	Variety	Date picked	Reducing sugars	Total sugar	Acid, as malic
			per cent	*per cent*	*per cent*
1	Derby Royal	June 2	1.58	5 30	1.40
		June 7	1.91	7.34	1.29
		June 12	2 42	7.82	1.06
1	Royal	June 2	2.25	5.59	1 59
		June 7	2.26	8.02	1.47
		June 12	2.63	8.62	1.16
2	Royal	June 17	1.77	5.47	1.41
		June 17	2.36	8.09	1.19
3	Royal	June 18	2.25	6.36	1.24
		June 18	3.00	8.65	1.03
		June 23	2.50	7.50	1.20
		June 23	2.75	8.46	1.05
		June 23	3.35	9.86	0.77

ACID CONTENT

Previous to 1930, acid determinations were made either on the extracted juice or the flesh of the fruit. Where the latter was used, 50 grams of the fruit tissue was extracted for several weeks in water covered with toluene and the solution titrated against N/10 sodium hydroxide using phenolpthalein. The method employed during 1930 was essentially the same as used previously except for the fact that an aliquot portion of the alcohol solution, remaining after the sugar extraction, was used. The results secured from this method may be slightly lower than those secured from the samples put up in water but they check with a high degree of accuracy. Table 24 gives the results on Gravenstein apples. Those secured from pears were very similar to those secured with apples. It would therefore seem unnecessary to put up the extra water sample for acid determinations.

TABLE 23

STARCH, SUGAR, AND ACID CHANGES IN GRAVENSTEIN APPLES DURING THE PERIOD OF HARVEST, HEALDSBURG-SEBASTOPOL DISTRICT; 1930

Orchard No.	Date picked	Starch: When picked	Starch: After 10 days at 50° F	Starch: After 10 days at 70° F	Reducing sugars: When picked	Reducing sugars: After 10 days at 50° F	Reducing sugars: After 10 days at 70° F	Total sugar: When picked	Total sugar: After 10 days at 50° F	Total sugar: After 10 days at 70° F	Acid: When picked	Acid: After 10 days at 50° F	Acid: After 10 days at 70° F
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	June 30	1.16	0.48	0.10	6.07	6.68	7.28	6.92	7.86	8 56	0.96	0.86	0.74
	July 14	1.12	0.46	0.21	6.63	6 74	8.54	7.60	8.42	10.27	0.80	0 77	0.73
	July 29	1.31	0 74	0.24	6.76	7.11	7 60	8.50	9.34	10 45	0.82	0.76	0.68
2	July 7	1.49	0.74	0.36	6 59	6 80	7.64	7 90	8 44	9.30	0 77	0 79	0.76
	July 28	1.00	0.32	0.26	7.25	7.69	8.44	9.70	10 07	10.62	0.76	0.68	0.66
3	July 7	1.40	0.52	0.30	5 98	6.77	7.42	7 29	8.34	8.78	0.82	0.77	0.70
	July 21	1.33	0.58	0.25	6.29	6.97	8 14	8.18	8 89	9.68	0 80	0.72	0.69
4	July 7	1.69	0.90	0.44	6.41	7.12	9.20	7 39	8 60	10.75	0.94	0.91	0.73
	July 21	1.33	0.56	......	7.78	8 76	.....	9.56	10.78	......	0.78	0.77	......
5	July 14	1.16	0.30	0.11	6.46	6.70	7.04	7.82	7.92	8.64	0.86	0.74	0.68
6	July 9	1.23	0.62	0.11	6.72	7.43	7.54	8.07	9 41	9.31	0.87	0 80	0.69
Averages, July 21 to July 29		1.24	0.55	0.25	7.02	7.63	8.06	8 98	9.77	10 25	0.79	0.73	0.68
Averages, June 30 to July 7		1.43	0.88	0.30	6.26	6.84	7 88	7.37	8.31	9.35	0.87	0.83	0.73
Average loss or gain between pickings		−0.19			+0.76			+1 61			−0 08		

TABLE 24

COMPARATIVE DATA ON ALCOHOLIC AND WATER EXTRACTIONS FOR THE DETERMINATION OF ACID IN GRAVENSTEIN APPLES

Sample No.	Alcohol extraction	Water extraction	Difference
	per cent, malic	*per cent, malic*	*per cent*
1	0 76	0.77	0 01
2	0.77	0.77	0.00
3	0.79	0.82	0.03
4	0.75	0 75	0.00
5	0.74	0.76	0.02
6	0.94	0.94	0.00
7	0.91	0.93	0.02
8	0 73	0.74	0.01
9	0.62	0.64	0.02

Plums.—Plums contain from 0.5 to 2.0 or 3.0 per cent of acid depending upon the variety, the time of harvesting, and whether the determination is made upon the expressed juice or upon the flesh. In most cases there is a distinct decrease in the acidity of the fruit as it ripens, either previous to or following harvest. When computed upon the juice, the acidity decrease may be as much as 50 per cent of the total acid present. Analyses of the flesh may or may not show any acid decrease. The decrease in acid varies rather markedly with different varieties. Diamond shows a small acid change, both before and after harvesting, while Beauty and Wickson show a marked decrease in the acidity of the juice but a relatively small amount in the flesh. This is apparently due to the fact that the acid becomes localized in the flesh near the skin and around the pit, and the juice coming from the main portion of the flesh contains relatively much less acid. This condition is more noticeable as the fruit approaches full maturity on the tree or as it ripens following harvest.

Peaches.—The percentage of acid in the flesh of peaches usually varied between 0.5 and 1.0 per cent. Of the six varieties analyzed, Elberta gave the lowest average and Tuskena the highest. Nightingale, Addoms and Blake,[30] working with Elberta and Shipper Cling peaches, report an increase in acidity with development until the fruit reaches the soft-ripe stage. The data presented in table 20 show this was true in some of the earlier-picked samples but that in most cases there was a gradual decrease in acidity with maturity. Generally speaking it would appear that there is little significant change in the acid content. A small decrease continues following harvest but this does not reach its minimum until the fruit has passed its prime eating condition.

Apricots.—The acidity of apricots is greater than that found in peaches and is similar in amount to that of most varieties of plums. There is a gradual and consistent decrease in acid content as the fruit matures on the tree (table 22) and a further decline, although somewhat less marked, after the fruit has been held for 10 days under a temperature of 50° F.

Pears.—Pears show a low percentage of acid which, according to table 21, varies rather widely between two successive years. Although the orchards from which the samples were taken in 1926 were not the same as those from which fruit was obtained the year previous, results of the analyses strongly suggest a considerable fluctuation in the acid content from year to year.

Changes in acidity slightly in advance of and during the commercial harvest season are confined within a narrow range. The fruit from the Sacramento River district behaved differently from that grown in the Sierra foothills, the former showing either no consistent change or a slight decrease in acid as the season advanced, while the latter shows a rather definite and consistent increase. Magness,[21] comparing samples from the Sacramento River district with those from Medford, Oregon, and Yakima, Washington, found fruit from the latter districts to show a slight gain in acids as the picking season advanced.

After ripening at 70° F samples from the Sacramento River showed a very slight change in acids with no consistent trend either up or down. Fruit from the Sierra foothills decreased slightly in acid content.

From the results thus obtained to date it would appear that the acid content and acid changes in pears are, within narrow limits, subject to considerable variation.

Apples.—Acidity in Gravenstein apples (table 23) shows a slight decrease within a two-weeks harvesting period and also a further decline after 10 days at 50° and at 70° F. Magness and Diehl[22] suggest that the decrease in acidity noted before harvesting is due to a dilution as the fruit increases in size (as illustrated in table 3). St. John and Morris,[35] as a result of several seasons' investigations with Jonathan apples state that the level of sugars, acids, and other fractions vary in different years and with the location of the individual fruits on the tree. In view of these variations they think it doubtful whether much significance can be attached to small changes in the quantity of acid.

Accepting these views as correct, the decrease in acid following removal of the fruit from the tree can scarcely be interpreted other than as an actual loss—the rate of decrease being greater after 10 days at 70° F than after the same period at 50°.

STARCH DETERMINATIONS

Determinations for starch were made only in the case of the apple. The residue from the alcohol extraction of the sugars was dried at 65° C for 24 hours, ground in a mortar, transferred to a drying dish, and returned to the vacuum oven at 65° C for 3 days. The samples were then transferred to ball-mill jars and ground for 20 to 22 hours. After adding 30 cc of distilled water the jars were placed in a water bath and held 30 minutes at 100° C. As soon as cool, 10 cc of taka-diastase solution (2 grams in 100 cc of water), 10 cc of sodium acetate buffer (pH 5.0), and 2 cc of toluene were added to each sample. Following incubation at 32° C for 18 hours the samples were washed from the ball-mill jars into centrifuge bottles, 5 cc of lead acetate added, and centrifuged. The lead was subsequently removed with a balanced amount of potassium oxalate, filtered and made up to 250 cc volume. From this, 100 cc samples were withdrawn and placed in 300 cc Erlenmeyer flasks, 10 cc of HCl (sp. gr. 1.125) added, and then refluxed on a steam bath for 2.5 hours. After neutralizing with 35 per cent NaOH and making up to 200 cc the samples were analyzed for glucose in the same manner as for reducing sugars. After subtracting the taka-diastase blank the amount of starch is expressed as glucose in percentage of fresh weight.

The starch content of Gravenstein apples is presented in connection with the data on sugars and acid in table 23. Except for one sample, starch showed a decrease as the picking season advanced while a very material reduction was noted after harvesting and holding the fruit for 10 days at 50° and at 70° F.

MATURITY CHANGES AS MEASURED BY ELECTRICAL CONDUCTIVITY

Winkler,[40] measuring permeability changes by recording the resistance of the tissues to the passage of a weak electric current, found that Yellow Newtown apples near the end of their storage period showed a marked increase in permeability. The flesh of the fruit beginning to show storage breakdown or internal browning had a much lower resistance than that still in normal condition. Unpublished work of Latimer[19] also shows that as the flesh of pears begins to break down there is a marked loss in resistance of the tissues. This, however, was preceded by an increase in resistance during development.

To further test the resistance of the flesh to an electrical current as a possible maturity index for harvesting Bartlett pears, samples of this fruit were collected at intervals from four orchards in widely separated districts and resistance determinations made at the time of harvesting, after a shipping period of 12 days at 50° F, and again when fully ripe.

Resistance measurements were taken in the usual manner with a Wheatstone bridge. The potential resistance of the bridge was balanced in the resistance box so as to have the bridge readings of equal sound intensity at 15–25 points each side of the center. The electrodes were frequently standardized in N/50 KCl solution at 25° C and all readings calculated as specific resistance at this temperature. Temperatures of some samples of fruit varied slightly from this mean but those run at comparable temperatures show that variations not due to temperature are many times those which may be caused by a fluctuation of several degrees.

Heavy platinum electrodes, 7.5 x 7.5 mm spaced 5 mm apart were suitably mounted so that readings could be made by inserting the electrodes directly into the flesh of the fruit. Readings were taken on opposite sides of each of ten specimens. Determinations were made (a) in the cortex area by forcing the electrodes through the skin of the fruit at the point of largest cross section diameter; (b) in the cortex area, forcing the electrodes into the flesh at right angles to the face of the cut cross section; and (c) in the pith area at right angles to the face of the cross section. A comparison of the readings secured in those positions is shown in table 25.

Readings (a) and (b) taken in the cortex area are very similar, the skin in most instances adding slightly to the resistance. Readings

in the pith area were found to be approximately only two-thirds those of the outer parts of the flesh. As pears first show physiological breakdown in this region these lower resistance readings might be construed as representing a more mature condition of the flesh in this part of the fruit. This, however, can scarcely be true since this differential, which remains fairly constant, exists previous to harvesting for commercial shipment. The readings do not increase with the later picked fruit which is considerably softer.

TABLE 25

SPECIFIC RESISTANCE OF THE FLESH OF BARTLETT PEARS; 1927

Locality	Date picked*	Specific resistance, ohms: When picked (a)†	When picked (b)	When picked (c)	After 12 days at 50° F (a)	After 12 days at 50° F (b)	After 12 days at 50° F (c)
Sacramento River district	June 20	5333	5340	3905	....	....	....
	July 6	5714	5372	3454	6150	6051	4083
	July 19	5879	5198	3444	6736	6400	4000
	August 6	6912	6625	4575	7440	7099	4859
Newcastle	July 1	4957	4868	3250	5195	4814	3386
	July 20	5969	5297	3624	6808	6864	4096
	August 3	6802	5955	3660	6246	6584	4097
	August 25	5580	5490	3945	6182	6150	4025
Santa Clara	July 13	4896	4637	3612	6646	5947	4322
	July 27	6112	5490	4035	6826	6101	4266
	August 10	6858	6617	4701	6352	5235	3630
	August 23	5910	6165	4350	6930	6345	4425
Placerville	July 14	4447	4229	3223	6660	6375	4665
	August 3	6187	6045	3045	6681	6923	4347
	August 17	6304	6400	4144	6787	6280	4687
	September 1	6030	5820	3870	....	....	....

* The four dates in each case represent respectively 14–20 days previous to commercial harvest; early commercial picking; midseason picking; and last of crop.

† Readings taken (a) in cortex area through the skin of the fruit; (b) in cortex area, cut cross section; (c) in pith area, cut cross section.

Further comparison between softening and specific resistance readings obtained at (a) on the same fruits at different dates previous to and during the commercial picking season are illustrated in figure 1. The resistance curves show a distinct rise throughout most of the picking season, with a slight lowering of resistance in late-picked fruit. While this lower resistance accompanies softening it is apparently independent of it as all samples picked at the end of the commercial harvest season had a higher resistance than comparable samples at the beginning of the season. This is in agreement with

the general idea of all permeability, in that changes in resistance are due to the solution of electrolytes within the cell rather than to changes in the cell walls, the latter being considered as offering little or no resistance to the passage of ions.

Latimer[19] states that, "during the growing period the resistance of the tissue increased gradually until the fruits reached their maximum size and were morphologically mature." Although it has been shown that the fruit continues to increase in size as long as usually allowed to remain on the tree the statement of Latimer is generally substantiated by the resistance curves in figure 1. A comparison of

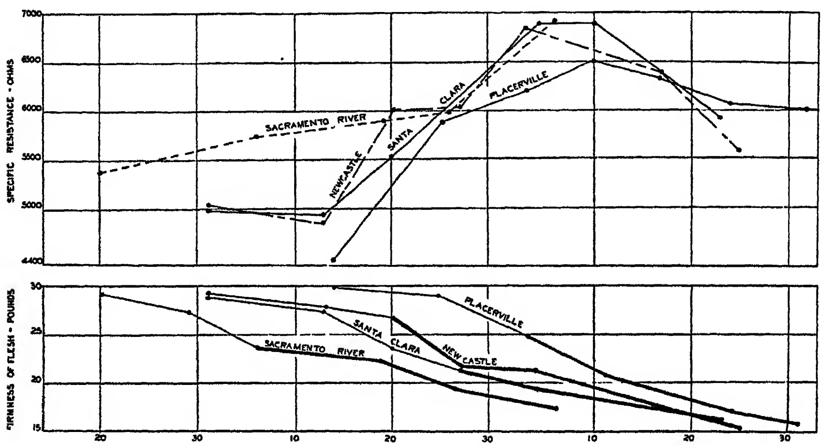

Fig. 1. Softening and specific resistance of Bartlett pears, 1927. Heavy lines represent period of commercial harvest.

the resistance curves with those of softening, show that, instead of any direct correlation existing between resistance and softening, up to full development of the fruit the two changes are to a certain extent inversely proportional. With late-picked fruit the resistance decreases and has a tendency to drop with the softening of the flesh.

Previous investigations have shown a general correlation between the quality of Bartlett pears and their firmness when picked. A pressure test of 22 pounds in the Santa Clara Valley and of 23 to 25 pounds in the interior valleys has been recommended as the maximum firmness at which the fruit should be harvested and expected to ripen with fair quality. To secure the *best* quality, harvesting in most sections should be delayed until the pressure drops to 20 pounds.

Noting the dates on the lower part of figure 1 when the fruit from each of the four districts had softened to these pressures, and then

observing the resistance of the fruit on the same days, it is seen that with a firmness of 22–23 pounds the resistance shown by the fruit from the different sections is rather variable (table 26). With a drop in firmness to 20 pounds, this variation becomes less in three out of the four samples and it might appear that a resistance of 6450 to 6600 ohms does mark the beginning of the period when pears from the Newcastle, Placerville, and Santa Clara Valley ripened with *good* quality.

TABLE 26

Specific Resistance of Bartlett Pears at the Time of Commercial Harvest; 1927

Locality	Date testing 23 pounds pressure	Specific resistance, ohms	Date testing 20 pounds pressure	Specific resistance, ohms
Sacramento River district	July 11	5750	July 25	5950
Newcastle	July 25	6000	August 8	6650
Placerville	August 7	6400	August 13	6450
Santa Clara Valley	July 25	5950*	August 1	6600

* At a pressure of 22 pounds.

The dessert quality in the later pickings of fruit after the resistance readings had begun to drop were, however, as good if not superior to that in the fruit picked when showing its highest resistance.

Pears from the Sacramento River district possessed good quality considerably before reaching their maximum resistance and all fruit from the orchard under test was harvested before the resistance declined. While therefore the general rise in resistance may be correlated with general maturity it is too variable to be considered as a picking index.

The time of the initial commercial picking in each of the four orchards is shown in figure 1. In the Sacramento River district, at Newcastle, and at Placerville, harvesting began as soon as the fruit was 2¼ inches in diameter and from 3 to 5 days before it would meet a 23-pound pressure test. In the Santa Clara orchard the first picking of fruit was delayed until it tested 21 pounds and was 2½ inches or over in size.

The specific resistance of the fruit after storing for 12 days at 50° F, or what might be considered the time the fruit is in transit to eastern markets is, in most cases, slightly higher than when harvested. However, measurements taken on late-picked fruit from the Santa Clara Valley may show a marked decrease in resistance (fig. 2).

After removing to a temperature of 70° F following a 12-day period at 50°, Bartlett pears reach prime eating condition in from

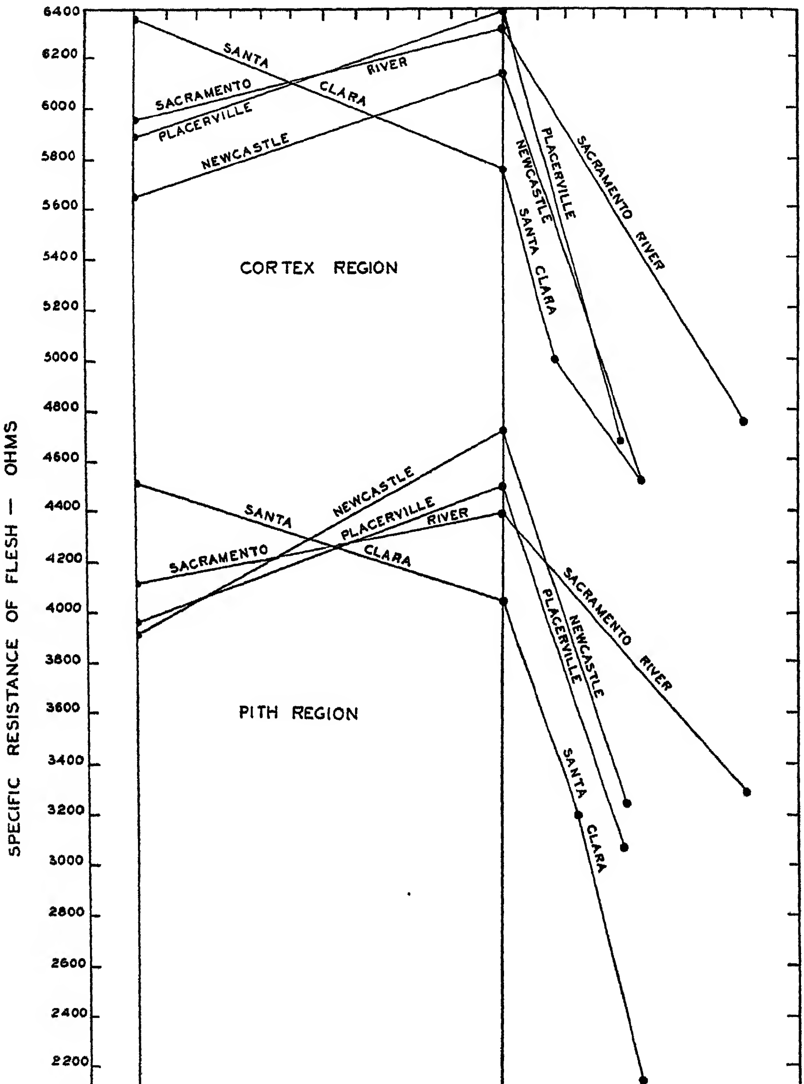

Fig. 2. Specific resistance readings of the flesh of late-picked Bartlett pears from the time of harvesting until ripe. Top, cortex region; bottom, pith region.

2 to 5 days. During this ripening period, and as the fruit becomes overripe and shows evidence of physiological decay, the resistance of the tissues falls very rapidly. Figure 2 illustrates these changes as measured in both the cortex and pith areas of certain samples of late-picked fruit.

The results are in agreement with those of Winkler[40] and of Latimer,[19] and indicate rather clearly that in most instances the resistance of Bartlett pears maintains an upward trend until the fruit approaches edible condition and then decreases rapidly as the flesh becomes soft.

INFLUENCE OF ETHYLENE GAS ON THE RIPENING OF GRAVENSTEIN APPLES

The Gravenstein apple is the earliest commercial variety grown in California for eating out of hand but as harvested in this state it arrives on the eastern markets considerably in advance of the time when it is most attractive as a dessert apple. The success which has been attained by a number of investigators in hastening the coloring and ripening of certain fruits and vegetables by the use of ethylene gas suggested its possibilities in hastening the ripening of the Gravenstein apple and also of a number of varieties of fall and winter pears.

Box lots of apples were secured from six different orchards in the Sebastopol section, pickings being made early in the shipping season and again two to four weeks later. All fruit was brought to Davis the second day following harvest and immediately treated or placed under storage conditions. Ethylene gas was used at a strength of 1–1000, the fruit being placed in tight chambers of 20–25-box capacities and under temperatures of 50° and 70° F. Runs extended for 4 and for 10 days, the chambers being opened and regassed each second day. Following the period of treatment the samples were exposed to a temperature of 65°–75° F for ripening.

Influence on Color.—Notations on color were made at the time of picking and also after treating by comparing the ground color of the fruit with a color chart showing different shades of green to yellow.[2] Although color changes were not always consistent, there is no doubt but that the treatment influenced some samples, changing the color

[2] The chart used is that adopted by the California State Department of Agriculture in the shipping of Bartlett pears, reproduced in California Experiment Station Bulletin 470, "Maturity Standards for Harvesting Bartlett Pears for Eastern Shipment," and similar to that used by Magness and others in U. S. D. A. Dept. Bul. 1406, "The Ripening, Storage and Handling of Apples."

of the skin from a decided light green to a greenish yellow. This change, however, was apparent in only 35 per cent of the samples held at 50° F and in a little over 50 per cent of those held at 70° F. Early-picked fruit responded in a greater number of cases than did that picked later.

In certain instances the changes were practically as marked after 4 days of treatment, at 70° F plus 6 days ripening in air, as they were after 10 days gas treatment at the same temperature. As was anticipated, temperature had a decided influence on the rate of yellowing. In no instance was there any effect upon the red color. Fruit wrapped and packed showed practically the same color change as that unwrapped.

TABLE 27

INFLUENCE OF ETHYLENE GAS UPON THE SOFTENING OF GRAVENSTEIN APPLES; 1930

Orchard No.	Date picked	Firmness when picked	Firmness of fruit after 10 days at			
			50° F		70° F	
			Check	Treated	Check	Treated
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
1	June 30	19.7	....	....	13.9	10.6
	July 29	19.5	16.6	16 3	14 0	12.4
2	June 30	17.3	....	....	11.1	9.9
	July 21	15.4	13.7	13.4	13 5	11.1
3	July 7	17.0	17.8	16.2	17.3	11 0
	July 21	17.6	16.9	16.2	14.4	12.4
4	July 8	17.0	17.2	15.4	13.5	11.1
	July 21	17.8	15 6	14.3	15.6	9.5
5	July 7	17.4	17.8	17.4	16.4	12.1
	July 28	17.9	15.2	14.4	12.9	12.5
6	July 8	16.7	19.0	16.1	15.2	11.4
	July 21	18.2	16.6	16.0	14.2	13.8
Average		....	16.6	15.5	14.6	11.5
Average decrease		....	....	1.1	....	3.1

Influence on Softening.—Pressure tests taken when the fruit was picked and after it had been held 10 days at 50° F showed a difference of only 1 pound between the treated and untreated samples. When held at 70° F the difference averaged 3 pounds and was the most outstanding effect of the treatment (table 27). A rearrangement of the data in table 27 is presented in table 28 and gives more clearly the comparative differences between the softening of early and of

TABLE 28

INFLUENCE OF ETHYLENE GAS UPON THE SOFTENING AND RIPENING OF EARLY AND OF LATE-PICKED GRAVENSTEIN APPLES; 1930

Orchard No.	Date picked	Temperature at which the fruit was held, degrees Fahrenheit	Average firmness after 10 days, pounds		Additional number of days to ripen after 10 days' treatment or holding	
			Check	Treated	Check	Treated
		Early pickings				
1	June 30	50	....	....	14	13
		70	13.9	10.6	9	9
2	June 30	50	....	....	13	12
		70	11.1	9.9	9	9
3	July 7	50	17.8	16.2	10	9
		70	17.3	11.0	5	4
4	July 8	50	19 7	15 4	10	7
		70	13 5	11.1	6	5
5	July 7	50	17.8	17.4	10	8
		70	16.4	12.1	6	5
6	July 8	50	19.0	16.1	12	11
		70	15.2	11.4	6	4
Average		....	16.1	13.1	9.2	8.0
Average decrease		....	......	3.0	....	1.2
		Late pickings				
1	July 29	50	16 6	16.3	2	1
		70	14.0	12.4	2	2
2	July 21	50	13.7	13.4	8	8
		70	13.5	11.1	1	1
3	July 21	50	16.9	16.2	9	9
		70	14.4	12 4	9	9
4	July 21	50	15.6	14.3	8	8
		70	15.6	9.5	2	1
5	July 28	50	15.2	14.4	6	6
		70	12.9	12.5	3	3
6	July 21	50	16.6	16.0	9	9
		70	14.2	13.8	2	2
Average		....	14.9	13.5	5.1	4.9
Average decrease		....	....	1.4	....	0.2

TABLE 29

INFLUENCE OF ETHYLENE GAS UPON THE STARCH, SUGAR, AND ACID CONTENT OF GRAVENSTEIN APPLES; 1930

Orchard No.	Date picked	When picked				Treatment	After 10 days at 50° F				After 10 days at 70° F			
		Starch as glucose	Reducing sugars	Total sugar	Acid, as malic		Starch as glucose	Reducing sugars	Total sugar	Acid, as malic	Starch as glucose	Reducing sugars	Total sugar	Acid, as malic
		Figures in per cent					*Figures in per cent*				*Figures in per cent*			
1	July 7	1.49	6.59	7.90	0.77	Check	0.74	6.80	8.44	0.79	0.32	7.64	9.30	0.76
						Treated	0.53	7.26	8.95	0.78	0.20	8.38	9.56	0.68
2	July 28	1.00	7.25	9.70	0.76	Check	0.36	7.69	10.07	0.68	0.26	8.44	10.62	0.66
						Treated	0.34	8.02	10.20	0.66	0.23	8.18	9.92	0.63
3	July 7	1.69	6.41	7.39	0.94	Check	0.90	7.12	8.60	0.91	0.44	9.20	10.75	0.73
						Treated	....	8.24	9.66	0.79	0.25	7.97	8.82	0.66
4	July 21	1.33	7.78	9.56	0.78	Check	0.56	8.76	10.78	0.77	....	....	....	....
						Treated	0.45	8.82	10.83	0.70	0.27	8.48	10.07	0.62
5	July 14	1.16	6.46	7.82	0.86	Check	0.30	6.70	7.92	0.74	0.11	7.04	8.64	0.68
						Treated	0.28	6.89	8.56	0.72	0.11	7.25	8.58	0.61
6	July 9	1.23	6.72	8.07	0.87	Check	0.62	7.43	9.41	0.80	0.11	7.54	9.31	0.69
						Treated	0.28	7.48	9.44	0.72	0.10	7.69	9.19	0.60
7	June 30	1.16	6.07	6.92	0.96	Check	0.48	6.68	7.86	0.86	0.10	7.28	8.56	0.74
						Treated	0.25	7.26	8.64	0.79	0.09	7.04	7.51	0.78
8	July 14	1.12	6.63	7.60	0.80	Check	0.46	6.74	8.42	0.77	0.21	8.54	10.27	0.73
						Treated	0.36	6.92	8.52	0.74	0.17	9.08	11.30	0.72
9	July 29	1.31	6.76	8.50	0.82	Check	0.74	7.11	9.34	0.76	0.24	7.60	10.45	0.68
						Treated	0.65	7.30	9.50	0.72	0.24	7.35	10.20	0.68
10	July 7	1.40	5.98	7.29	0.82	Check	0.52	6.77	8.34	0.77	0.30	7.42	8.78	0.70
						Treated	0.43	6.88	8.36	0.75	0.19	7.80	8.60	0.67
11	July 21	1.33	6.29	8.18	0.80	Check	0.58	6.97	8.89	0.72	0.25	8.14	9.68	0.69
						Treated	0.39	7.06	9.34	0.71	0.17	7.68	9.30	0.62
Average	....	1.29	6.63	8.08	0.83	Check	0.57	7.16	8.91	0.78	0.23	7.88	9.63	0.70
						Treated	0.39	7.46	9.27	0.73	0.18	7.90	9.37	0.66

late-picked fruit. With the former the average difference between the treated and check samples was 3 pounds while with the latter it was only 1.4 pounds. This softening is reflected in the time required for each sample to reach its prime eating condition. In each case the number of days recorded represents the first day when the fruit seemed to have reached its best quality. Obviously, the recording of such data presents opportunity for error and is less satisfactory than where possible to use a definite standard of measurement. On the average, however, the early-picked fruit which was treated ripened one day ahead of that not treated. Late-picked samples showed no difference in this respect.

Influence on Dessert Quality and Chemical Composition.—Critical sampling for dessert quality seems to justify the statement that in two or three lots only, was the treated fruit noticeably sweeter and more mellow in texture than that of the untreated. Results of chemical analysis of treated and untreated samples are given in table 29. These data show the normal changes in starch, sugars, and acids during a ripening period of ten days at 50° and at 70° F, and also the changes induced by the ethylene gas treatment. All samples of fruit treated at 50° showed a slight increase in sugars and a decrease in acid and in starch over the check samples. Comparable lots treated at 70° F likewise showed a decrease in starch and in acids in 8 out of 11 samples. In most cases there was no increase in sugars. In fact most treated samples showed a decrease, possibly due to the use of these materials in a more rapid metabolism of the tissues.

Using the averages for starch, sugars, and acids given in table 29 as a basis, the per cent loss or gain in these substances is illustrated in figure 3. From this it is readily apparent that the greatest loss or gain was with the fruit held at 70° F but that the greatest differences between the treated and check samples occurred at 50°. At this temperature the percentage increase in total sugar averaged 4.5 per cent while the decrease in acid and starch was 6.0 and 13.8 per cent, respectively.

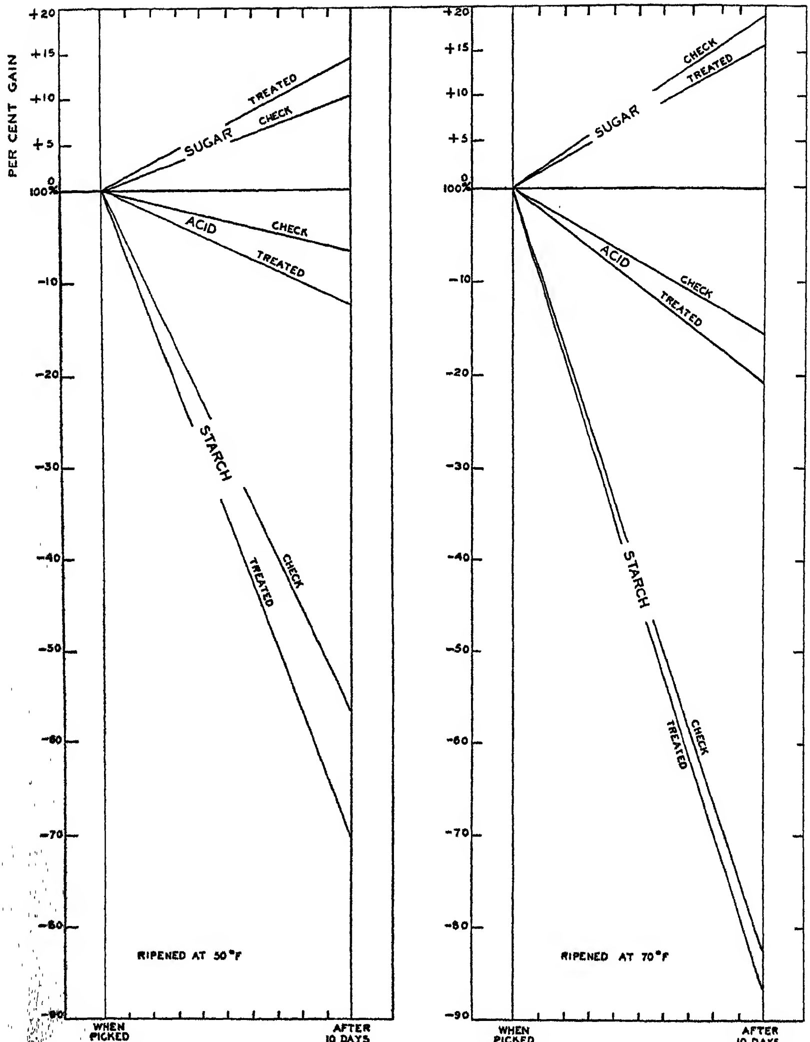

Fig. 3. Gain or loss in starch, sugar, and acid in Gravenstein apples after ten days ripening at 50° and 70° F. Figures are in percentage of the amounts present when the fruit was harvested.

INFLUENCE OF ETHYLENE GAS ON THE RIPENING OF PEARS

Methods of handling Anjou, Beurre Bosc, Beurre Clairgeau, Comice, Glou Morceau, Winter Nelis, Easter Beurre, and Kieffer pears were similar to those with apples. All samples, however, were held at 70° F during treatment and the duration of the treatment was reduced in most instances to a period of 3 or days. A few samples were treated immediately after harvesting, but in most cases the treatments followed a 10-day 'in transit' period during which time the fruit was held at 50° F. With the more important varieties, tests were also run after a storage period of from 12 to 18 weeks at 32° F.

Fruit was obtained primarily from the Santa Clara Valley with some samples from the Sacramento River and Sierra foothill districts. Influences of the gas on rate of ripening were confined to recording color changes, the number of days required for each sample to reach prime eating condition and its period of marketability. Chemical analyses were run on a number of samples of the Beurre Bosc and Winter Nelis varieties.

Anjou.—The influence of ethylene gas upon the ripening of Anjou pears is shown in table 30. When the fruit was held continuously at 70° following harvest the ethylene treatment reduced the period of ripening in the two samples from 17 and 26 days to 10 days. Comparable samples from both Newcastle and Davis held at 50° for 10 days and then treated showed an average difference in ripening of 1.3 days. Samples stored for 12 weeks at 32° F previous to treatment showed the same difference.

Untreated pears from the Santa Clara Valley were exceedingly slow in reaching a ripe condition; in fact the flesh became tough and ripened only imperfectly after 3 to 5 weeks. Treated samples ripened very satisfactorily in 9 to 11 days and with better quality than the untreated samples. Slight to rather marked differences in color were noted in about half of the samples treated. These differences were noticeable in from 4 to 8 days.

Beurre Bosc.—Samples of Beurre Bosc pears from two orchards in the Santa Clara Valley and in the Sacramento River district were treated and held at 70° F immediately following harvest. Fruit from the Santa Clara Valley ripened somewhat slower than that from the River district. In both instances, however, the ethylene treatment

hastened the process, the treated samples from the former section ripening in 11.3 days or 8 days previous to that of the untreated samples. In the latter district the time was 11.5 and 14.5 days (table

TABLE 30

INFLUENCE OF ETHYLENE GAS UPON THE RIPENING OF ANJOU PEARS; 1930

Locality	Orchard No.	Date picked	Time treated	Days to ripen, including days treated	Color and quality when ripe
Treated as soon as harvested					
Newcastle	1	August 15	Treated 2 days	10	Yellow, good
			Check	26	Greenish yellow, poor, shriveled
	2	August 15	Treated 2 days	10	Greenish yellow, fair to good
			Check	17	Greenish yellow, fair to good
Treated after storing 10 days at 50° F					
Newcastle	1	August 15	Treated 2 days	6	Yellow, good
			Check	6	Yellow, good
	2	August 15	Treated 2.5dys.	5	Yellow, fair to good
			Check	7	Yellow, fair to good
Davis		August 15	Treated 3 days	11	Yellow, good
			Check	13	Greenish yellow, good
Santa Clara	1	August 22	Treated 3 days	11	Greenish yellow, good
			Check	33	Greenishyellow, poor, shriveled
	2	August 30	Treated 4 days	9	Yellow, very good
			Check	25	Greenish yellow, good, but fruit slightly shriveled
	3	August 30	Treated 4 days	10	Yellow, good
			Check	35	Light yellow, fair to good
Treated after storing 12 weeks at 32° F					
Newcastle	1	August 15	Treated 3 days	10	Greenish yellow, good
			Check	11	Greenish yellow, fair, insipid
	2	August 15	Treated 4 days	12	Greenish yellow to yellow, good
			Check	13	Greenish yellow to yellow, good
Davis		August 15	Treated 3 days	11	Yellow, good
			Check	13	Greenish yellow, good

31). Color in each of the five treated samples was either developed or hastened by the use of the ethylene. The flesh of two untreated samples became somewhat tough and shriveled previous to ripening and the quality was not good. In the other two samples no difference in quality could be detected.

TABLE 31

INFLUENCE OF ETHYLENE GAS UPON THE RIPENING OF BEURRE BOSC PEARS; 1930

Locality	Orchard No.	Date picked	Time treated	Days to ripen, including days treated	Color changes
Treated as soon as harvested					
Santa Clara Valley	1	August 29	Treated 4 days	13	Color changing from olive green to greenish yellow after 6 days
			Check	20	Color remained olive green, fruit shriveled
	2	August 30	Treated 3 days	11	Golden yellow when ripe
			Check	16	Green after 11 days, becoming golden yellow when ripe
	3	August 29	Treated 3 days	10	Noticeable change in 3 days, golden yellow in 10 days
			Check	22	Green after 10 days
Sacramento River district	1	August 4	Treated 4 days	9	Fruit yellow when ripe
			Check	11	Fruit green and slightly shriveled when ripe
	2	August 4	Treated 4 days	14	Golden russet when ripe
			Check	18	Light yellow, russet, ripening unevenly and slightly shriveled on neck of the fruit
Treated after storing for 10 days at 50° F					
Santa Clara Valley	1	August 22	Treated 3 days	5	No apparent increase
			Check	5	
		August 30	Treated 4 days	9	No apparent increase
			Check	10	
	2	August 22	Treated 3 days	9	Noticeably more yellow after 3-4 days
			Check	10	
		August 30	Treated 4 days	11	Noticeably more yellow after 3-4 days
			Check	11	
	3	August 22	Treated 3 days	9	Noticeably more yellow after 3 days
			Check	12	
		August 30	Treated 4 days	11	No apparent increase
			Check	11	
	4	August 22	Treated 3 days	8	Noticeably more yellow after 3 days
			Check	10	
		August 30	Treated 4 days	10	No apparent increase
			Check	12	
	5	August 22	Treated 3 days	8	Noticeably more yellow after 3 days
			Check	10	
	6	August 22	Treated 3 days	8	No apparent increase
			Check	10	
Sacramento River district	1	August 13	Treated 2.5 d's	7	Possibly more yellow
			Check	7	
	2	August 13	Treated 2.5 d's	6	No apparent increase
			Check	6	
	3	August 13	Treated 2.5 d's	7	Noticeably more yellow after 3 days
			Check	7	
Penryn	—	August 15	Treated 2.5 d's	4	No apparent increase
			Check	4	
Newcastle	—	August 15	Treated 3 days	5	No apparent increase
			Check	5	
Auburn	—	September 3	Treated 4 days	6	No apparent increase
			Check	6	
Davis	—	August 29	Treated 4 days	10	Noticeably more yellow after 4 days
			Check	11	

Fruit from a larger number of orchards was treated after an initial storage period of 10 days at 50° F. Samples from the Santa Clara Valley again responded to the ethylene treatment but in a less degree than when treated as soon as harvested. Early-picked fruit showed a more uniform, or in some cases a greater response both in time of ripening and in coloring than did that picked a week following. Average differences in the time of ripening for the treated samples harvested August 22 (table 31) were 1.7 days. Only 1 of the 6 samples failed to show a noticeable difference in time of ripening. Increased color development was apparent in 4 out of 6 samples. No difference could be detected in quality.

Pears harvested August 30 from four of the above orchards failed to show consistent results, only 2, or half of the treated samples, showing a difference in rate of ripening with an average increase of 0.8 days. Only one sample showed any noticeable increase in color. No difference could be detected in quality.

Samples of Beurre Bosc from three orchards in the Sacramento River district and from a like number in the Sierra foothills failed to show any influence of the ethylene on the rate of ripening. All samples ripened within a week. Color differences were likewise noticed in only one or two cases, due in part at least to slightly more color development and a greater amount of russet on the fruit when harvested. A single sample of treated fruit from the University Farm at Davis ripened one day in advance of the untreated sample and showed more rapid color development.

Nine samples of Beurre Bosc from the Santa Clara Valley, one from the Sierra foothills and one from Davis, were also placed in 32° storage and held 10 weeks previous to exposure of ethylene gas. Treatment for 4 days increased the rate of ripening one day in 5 of the samples, the other showing no influence of the gas. Color differences were noted in 4 samples.

From the data secured it appears that ethylene is capable of hastening both softening and coloring of Beurre Bosc, as well as Anjou, but that its influence is greatest with early-picked fruit and that treated previous to storage. Except where the fruit was ripened immediately without the usual period of storage, there were only one or two cases where possibly the quality was improved by the use of ethylene.

Beurre Clairgeau.—Results with Beurre Clairgeau pears were again rather marked where the samples were treated immediately following harvest. Fruit treated after being stored for 10 days at

50° F showed a slight effect of the ethylene on two of the samples while the average difference in ripening with the fruit previously held at 32° was practically negligible. Slightly better flavor was noted in samples ripened immediately and in two of those ripened after the 10-day holding period. As Beurre Clairgeau is usually of a light greenish yellow to yellow color when harvested little color difference due to ethylene was noted.

Comice.—Only one sample of Comice grown in the Santa Clara Valley was subjected to the ethylene treatment. Treatment was given for 4 days following storage for 10 days at 50° F. The treated sample ripened in 8 days and the check sample in 10 days. At the end of the treatment period the treated sample was turning yellow while only a slight change of color had occurred in the check sample.

Glou Morceau.—This variety requires a relatively long ripening season and attempts to ripen two samples picked August 30 without holding in storage resulted in the fruit becoming tough and shriveled and with little change in color after 30 days. Concomitant lots treated with ethylene ripened rather imperfectly after 21 and 25 days and were of yellow color. Development of color was noticeable in 12 days. Two samples ripened after 10 days at 50° F showed practically no difference either in softening or color as a result of 6 days' treatment. Of five samples held at 32° for 16 weeks previous to 6 days' treatment only two ripened one day in advance of the checks. Both of these samples showed a noticeable increase in color.

Easter Beurre.—Attempts were made to ripen three samples of Easter Beurre immediately after harvesting, three samples after holding the fruit for 10 days at 50° F and two samples after holding for 18 weeks at 32° F. After being in storage at 32° the fruit required 10 and 20 days to ripen, the difference being due to its maturity when picked. Ethylene treatment for 4 days after removal from storage did not hasten ripening or produce any noticeable difference in color. The fruit which was not stored, as well as that stored for only 10 days, either failed to ripen or ripened very imperfectly, all specimens becoming shriveled and spongy with only slight change in color. Easter Beurre, which has excellent keeping quality in storage, also ripened very slowly when placed under ripening temperatures, and longer periods of treatment will be necessary to demonstrate the influence of ethylene with this variety.

Winter Nelis.—Untreated samples of Winter Nelis responded similarly to the Easter Beurre variety in that all attempts to ripen the fruit as soon as harvested resulted in failure. Comparable lots which

were treated ripened fairly satisfactorily. Of the three lots held 10 days at 50° F before being treated one sample showed no influence of ethylene, one treated sample ripened two days in advance of the check, while the third ripened six days earlier. After 15 weeks at a

TABLE 32

INFLUENCE OF ETHYLENE GAS UPON THE RIPENING OF WINTER NELIS PEARS; 1930

Locality	Date picked	Time treated	Days to ripen, including days treated	Color and quality when ripe
Treated as soon as harvested				
Santa Clara	August 22	Treated 2.5 d's	13	Fair quality
		Check	—*	
	August 30	Treated 3 days	12	Fair to good quality, yellow color
		Check	—*	Green and spongy
	August 30	Treated 3 days	12	Fair quality
		Check	—*	Green and spongy
	August 30	Treated 3 days	12	Fair quality
		Check	—*	
Davis	August 29	Treated 3 days	12	Fair to good quality, greenish yellow color
		Check	—*	Green and spongy
Treated after storing for 10 days at 50° F				
Santa Clara	August 30	Treated 4 days	11	No apparent increase, fair quality
		Check	11	
Davis	August 29	Treated 4 days	11	No apparent increase in color
		Check	17	
	September 9	Treated 4 days	9	No apparent increase in color
		Check	11	
Treated after storing for 15 weeks at 32° F				
Santa Clara	August 30	Treated 4 days	12	No apparent increase in color or firmness, fair quality
		Check	12	
Davis	August 29	Treated 4 days	12	No apparent increase in color or firmness, fair quality
		Check	12	

* Fruit failed to ripen in 30 days.

temperature of 32° all samples ripened in 12 days. The data are presented in table 32 and seem to justify the conclusion that ethylene is capable of ripening Winter Nelis within two weeks after harvesting. Untreated samples have subsequently been ripened successfully in 30 days under a temperature of 50° F.

Kieffer.—Only three samples of Kieffer were secured but the results obtained indicate that this variety responds readily to the use of ethylene gas. Although all samples, when fully ripe, had a very clear, deep yellow color, the rate of development of this color was markedly influenced by the ethylene treatments. Softening of the flesh was not marked as the variety attains its characteristic color, flavor, and aroma while still firm. However, differences in ripening were noted as shown in table 33. The quality of the early-picked samples was superior in the treated samples, the flesh being sweeter, more juicy and more aromatic.

TABLE 33

INFLUENCE OF ETHYLENE GAS UPON THE RIPENING OF KIEFFER PEARS; DAVIS, 1930

Date picked	Color when picked	Time treated	Days to ripen, including days treated	Color changes
Treated as soon as harvested				
August 29	Green	Treated 4 days	15	Yellowish green to light yellow after 4 days, yellow after 9 days
		Check	19	Yellowish green after 4 days, greenish yellow after 9 days
Treated after storing for 10 days at 50° F				
August 29	Green	Treated 4 days	9	Yellow after 4 days, golden yellow after 11 days
		Check	11	Light yellow after 4 days, yellow after 11 days
September 9	Yellowish green	Treated 4 days	7	Yellow after 4 days
		Check	8	Light yellow after 4 days

Influence of Ethylene Gas on Dessert Quality and Chemical Composition of Pears.—In some cases, as previously noted, treated samples of fruit were of slightly better quality than the untreated samples. Generally speaking, however, when the check samples ripened in a normal manner their quality was the same as that of the treated lots. A rather limited number of chemical analyses for sugars and acid show little difference as a result of the ethylene treatment. The averages shown in table 34 are the results of analyses on samples treated 4 days after the fruit had previously been in storage for 10 and 15 weeks. In view of the results on softening and color, it would appear that greater differences in chemical composition might have been found had samples been analyzed within a short time after harvesting.

TABLE 34

SUGAR AND ACID CONTENT OF TREATED AND UNTREATED BEURRE BOSC AND WINTER NELIS PEARS AFTER STORAGE PERIOD OF 10 AND 15 WEEKS, RESPECTIVELY, AT 32° F

Variety	Date picked	Reducing sugars		Total sugar		Acid, as malic	
		Treated	Check	Treated	Check	Treated	Check
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Beurre Bosc	August 22, 1930	6.13	6 13	8 23	8 29	0.22	0.22
	September 12, 1930	5.55	5.42	8.69	8.51	0.14	0.14
Winter Nelis	August 30, 1930	9.25	9 10	10.55	10.29	0.22	0.22

SUMMARY AND CONCLUSIONS

Measurements made on several varieties of plums, peaches, pears, and on Gravenstein apples show that these fruits continue to increase in size until more mature than they are allowed to become before harvesting for eastern shipment and that early harvesting for commercial shipment frequently results in a considerable loss of tonnage.

Although light may hasten their coloring, plums are able to develop their characteristic overcolor when sunlight is excluded. Plums showing only slight red or blue color when harvested may thus develop their full color while in transit to eastern markets, the extent and rapidity of the coloring being dependent upon the temperatures in transit.

Apples, peaches, pears, and apricots generally require sunlight for the development of their red color. Red color on these fruits therefore does not develop under storage conditions but may appear more noticeable after storage than when the fruit was harvested because of the change in ground color. This change from green to yellow may require only a few days in the case of the stone fruits, to a period of several weeks or months with late varieties of pears and apples stored under low temperatures.

Plums are a striking example of a fruit which may be harvested when showing only a trace of their overcolor and yet subsequently assume their full blue or red color after harvesting and while under transit or storage conditions.

Softening of the flesh is one of the most important ripening changes taking place in deciduous fruits and with the use of mechanical pressure testers can now be successfully measured. Fruits not only soften rapidly after beginnnig to change color, but tests with Bartlett pears have shown that softening may begin at a time when the fruit has attained less than half its full size.

When measured by a mechanical pressure tester different varieties of the same fruit show considerable variation in firmness of flesh and in its rate of softening. Climatic conditions and the type of rootstock upon which the tree is worked also have been found to be important factors in the softening of Bartlett pears. In general, however, the rate of softening immediately preceding and during harvest is from ½ to 1 pound per day with plums, peaches, and apricots; apples and fall pears ½ to 1½ pounds in ten days; and Bartlett pears 2 to 3 pounds in ten days.

As with coloring, softening of the fruit following its removal from the tree is primarily dependent upon the temperature at which it is held. Plums held at 43° F for 12 days softened approximately as much as comparable samples held at 52° F for only 6 days. Bartlett pears soften only 1 to 2 pounds in several months at 32° F, while they will ripen from two to three times as rapidly at 36° F. Somewhat similar differences were found with peaches and Gravenstein apples.

Plums, peaches, and pears all show an increase in soluble solids as the fruit ripens on the tree. The quantity of soluble solids is roughly twice that of the total sugars.

All of the fruits analyzed made a material gain in sugar content as the fruit colored on the tree. The stone fruits made a distinct gain in sucrose sugar, apples in both sucrose and reducing sugars, and pears primarily in reducing sugars. Apples and pears—usually harvested sometime in advance of eating ripe condition—show a decrease in starch and a marked gain in sugar content after picking.

Plums show an acid content of from 0.5 to 3.0 per cent, apricots approximately 1.0 per cent, peaches and apples 0.5 to 1.0 per cent, and pears 0.25 to 0.75 per cent. During maturity on the tree the acid of apricots, apples, and most plums and peaches shows a decrease. With some peaches and with Barlett pears from the Sierra foothill districts there may be an increase. The acid changes in peaches, Gravenstein apples, and pears are much less than in plums or apricots.

A comparison of the softening of Bartlett pears with the resistance of the tissues to the passage of an electrical current shows that while the fruit is maturing and softening on the tree, the specific resistance continues to show a distinct rise until late in the picking season. The samples tested show considerable variation in their resistance readings. While highest dessert quality is associated with high electrical resistance, additional work is considered necessary before the exact value of resistance readings as a picking index can be stated.

Specific resistance readings taken in the pith area of the pears were approximately only two-thirds as high as those secured in the cortex region.

After harvesting there is usually a continued rise in the resistance of the fruit until the flesh becomes overripe or shows signs of physiological decay. The resistance then drops rapidly.

Treating Gravenstein apples with ethylene gas can scarcely be recommended for commercial shipments although it resulted in hastening the softening and in increasing the yellow color of some samples. The difference in softening and coloring between the treated and untreated lots was most marked in the early-picked fruit and in those samples treated at a temperature of 70° F. When treated under a temperature of 50° F rather noticeable differences were also found in the starch, sugar, and acid content of the fruit.

Ethylene is also capable of both increasing the color and hastening the ripening of pears. The results, however, vary with different varieties and were not entirely consistent with all samples of the same variety. Early-picked fruit and that treated previous to storage showed the most striking differences.

Although a few of the treated samples of both apples and pears were judged as being of better dessert quality than the check lots, it was usually difficult to detect differences in flavor between the treated and the untreated lots. Chemical analyses of Gravenstein apple samples show that fruit which was treated with ethylene contained somewhat less starch and acid than that not treated. There was a slight increase of sugar in the samples treated under a refrigerator car temperature of 50° but slightly less sugar in most samples when treated under a temperature of 70° F. A limited number of analyses of Beurre Bosc and Winter Nelis pears show the ethylene treatment to have had practically no effect upon the sugar or acid content of the fruit.

ACKNOWLEDGMENTS

The author wishes to acknowledge the cooperation of Dr. J. R. Magness of the United States Department of Agriculture in the growth and firmness studies during the years of 1925 and 1926. L. R. McKinnon, of the Division of Pomology, has rendered material assistance in connection with the electrical resistance tests and the ethylene gas treatments. Chemical analyses during 1930 were made by E. A. Wilkins and Lawrence Newsome, under the supervision of O. Lilleland, while in previous years this work was largely performed by H. W. Allinger and F. G. Anderssen.

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THE STRUCTURE AND DEVELOPMENT OF FLOWERS IN FICUS CARICA L.[1]

IRA J. CONDIT[2]

INTRODUCTION

The botanical and horticultural literature of the cultivated fig is voluminous and had its beginning in ancient times. Some ancient writers treated such subjects as caprification, varieties, and pests in greater or less detail. Recent European investigators have added materially to our knowledge of fig morphology and the peculiar relations existing between the fig, the caprifig, and the pollinating insect.

Perusal of the literature, however, reveals many discrepancies and gaps in our knowledge of the structure and development of fig flowers. Accounts of the development of the macrogametophyte are very incomplete and practically nothing is found concerning the development of the microgametophyte. It is the purpose, therefore, of this paper to cover as completely as possible the detailed floral morphology of *Ficus carica,* both by description and by illustration. This may help to prevent in the future such confused accounts as are now current in botanical and horticultural textbooks written by authors who are not personally familiar with the peculiar life history of the fig.

Before proceeding with the morphological details it may be well to review briefly some of the facts regarding the nature of the fig and the caprifig and the habits of the pollinating insect. Such facts are of common knowledge to growers of Smyrna figs and to practical horticulturists in fig districts but are somewhat shrouded in mystery or lacking in clarity to many others.

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GENERAL MORPHOLOGY OF THE FIG

The genus *Ficus*, to which the edible fig (*F. carica* L.) belongs, is characterized by having its flowers borne inside a hollow receptacle or syconium. Horticulturally speaking, the fruit called the fig is the fleshy receptacle including the flowers; botanically speaking, the true fruits are the achenes within the syconium. To avoid confusion between horticultural and botanical terminology, the mature receptacle, the 'fruit' of the horticulturist, will be called the *syconium* in this paper.

In the syconia of all figs there is an apical ostiolum or eye which is usually more or less closed by scales. Within certain syconia of most if not all species of *Ficus* are various species of hymenopterous insects, the larvae of which develop from egg to adult inside the individual achenes. A fig flower the ovary of which contains the larva of a fig wasp is termed a gall flower. When the fig wasp emerges from the gall flower and leaves the syconium, it may be dusted with pollen if the staminate flowers near the ostiolum are mature at that time. Such pollen is carried to other figs which the insect enters to oviposit, and pollination is thus unwittingly accomplished.

Types of the Fig and Differentiation of Fruit Buds.—There are four general horticultural types of *Ficus carica*. The most primitive of these, the *caprifig*, has ovulate flowers with short-styled pistils (fig. 1*B*) and in many syconia staminate flowers also; the other three types (the *Smyrna, White San Pedro,* and the *common*), have ovulate flowers with long-styled pistils but have no staminate flowers.

Trees of the caprifig type charactistically produce three series of fruit buds each growing season. The first series of buds gives rise to the *profichi* or spring crop, the second series to the *mammoni* or summer crop, and the third series to the *mamme* or winter crop. The number of the series of fruit buds, however, depends upon climatic conditions. In the cool climate of Berkeley, California, and of southern France (Leclerc du Sablon, 1908*a*) the development of syconia is retarded, and only two series of buds mature, the mammoni crop being omitted. In the warmer climate of the Fresno district in California, the development is more rapid, and a second mammoni crop appears in late summer. In the extremely hot climate of Imperial Valley, California, there are seven more or less distinct generations of the blastophaga developing in as many crops of the caprifig tree (Condit, 1922).

Trees of the three fig types bearing long-styled flowers (Smyrna, White San Pedro, and common) have the first series of buds maturing into a *breba* crop (in Italy called the *fiori*) corresponding to the profichi of the caprifig; the second series of buds develops into the main crop (*pedagnuoli* or *forniti*), corresponding to the mammoni of the caprifig. Fig trees of the common type occasionally produce a third series of buds (*cimaruoli*), the syconia of which may mature the same season, may be destroyed by frost, or may remain dormant during the winter and mature the following spring. The development of these syconia depends upon the horticultural variety, the temperature, and the length of the growing season. Trees of the Kadota and Brown Turkey varieties are especially prolific and syconia continue to develop until the cooler weather of fall induces dormancy.

The buds in the axils of leaves are not macroscopically evident until the internodes have ceased elongating (Condit, 1926). Some varieties have one or frequently two fruit buds and a vegetative bud axillary to nearly every leaf. Other varieties have the fruit buds more scattered and borne in the axils of occasional leaves only.

No perfect hermaphroditic flowers have as yet been reported in receptacles of *Ficus carica* L. The flowers are fundamentally pistillate or staminate as the case may be, but rudimentary pistils are sometimes found in staminate flowers and rudimentary stamens in pistillate flowers.

The Caprifig Type and the Blastophaga.—The caprifig indigenous to southwestern Asia is the primitive type of cultivated fig, and the other three types of edible figs have undoubtedly evolved from it. The short-styled flowers of caprifigs are morphologically adapted to oviposition by the fig wasp (*Blastophaga psenes* L.), and syconia of each of the three crops of the caprifig tree harbor the larvae, pupae, and temporarily the adults of this insect. The presence of the immature blastophagas in mammoni and mamme figs is usually essential to their proper development on the tree. In some horticultural varieties of the caprifig, however, the profichi crop maturing in May or June consists of two kinds of syconia, those with and those without larvae in the gall flowers. Syconia inhabited by blastophagas normally keep green and plump until maturity and are designated as "insectiferous" figs (Eisen, 1896). On the same tree other syconia not inhabited by blastophagas develop parthenocarpically with an abundance of pollen and are known as "polleniferous" figs or "blanks." The latter are of no horticultural value, since the blastophagas are not present to distribute the pollen.

The seasonal history of the blastophaga in the San Joaquin Valley of California is as follows: The larvae hibernate in syconia of the mamme crop during the winter, transform to pupae in March; the adult blastophagas emerge early in April and oviposit in syconia of the profichi crop. Eggs laid in the profichi develop into adult blastophagas that oviposit in syconia of the mammoni crop early in June. Eggs laid in the mammoni figs early in June have, by the end of July, developed into mature blastophagas which oviposit in syconia of the second mammoni crop. These second mammoni figs may mature during late summer or early fall or may persist on the trees and constitute the mamme crop from which adult blastophagas emerge the next spring.

The manner in which the blastophaga lays eggs and the subsequent development of the larvae have a distinct morphological significance in the life history of the fig (Solms-Laubach, 1882; Leclerc du Sablon, 1908*b*; Longo, 1909). During oviposition the blastophaga pushes its ovipositor through the stylar canal almost to its base and then directly through the lower part of the style into the ovule. Usually only one egg is deposited in each ovule. Leclerc du Sablon (1907) occasionally found two eggs or larvae in the same ovule, and one of my preparations shows three very young larvae developing side by side next to the nucellus.

The blastophaga egg is extruded between the inner integument and the nucellus (fig. 1*B*). This is invariably the position of the egg in my preparations, although in a few cases some nucellar cells are found to have been punctured by the tip of the ovipositor. Ravasini (1911) and Longo (1912*b*) report finding an egg occasionally within the nucellus, but Grandi (1929) never found one in such a location. Leclerc du Sablon (1908*b*) states that the egg is deposited more or less deeply in the nucellar tissue with a resultant enlargement of the nucellus due to a stimulation of cell division and an increase in cell size. My observations show a growth in size of the nucellus following oviposition, but this is due to an increase in size of cells by vacuolation rather than to an increase in their number. The swelling of the blastophaga egg after its extrusion from the ovipositor produces a pronounced concavity in the surface of the nucellus. The young larva first feeds upon the adjacent nucellar cells but later subsists upon the endosperm.

Oviposition and larval development are correlated with purplish or violet coloration of floral pedicels and perianth in syconia of some horticultural varieties of the caprifig, such as the Milco and Samson (Condit, 1922). In other varieties of the caprifig, such as the

Stanford, and in all caprifigs of *Ficus pseudocarica* Miq. and *F. palmata* Forsk, thus far observed, the pedicels and perianth lobes are white or greenish yellow in all stages of gall-flower development.

The caprifig is of no economic value except to perpetuate the blastophaga and to produce the pollen necessary to pollinate the flowers borne by figs of the Smyrna type. Pollination of the flowers of Smyrna figs with pollen carried to them by blastophagas from the profichi crop of caprifigs is called caprification. Man modifies the normal life history of the blastophaga by placing mature profichi of the caprifig in fig trees of the Smyrna type, thus causing pollen-dusted wasps to enter syconia of Smyrna figs instead of caprifigs. The female blastophagas, which generally lose their wings as they push their way between the scales of the ostiolum, crawl over the stigmas of the long-styled flowers in a vain attempt to oviposit. Thus, although there is no oviposition, there is pollination and a consequent fertilization and maturation of achenes in the Smyrna fig. Eventually the wasp emerges from or dies within the syconium.

Some syconia bearing short-styled pistillate flowers also bear separate and distinct staminate flowers. Such staminate flowers are abundant at the apical end of syconia of the profichi crop but are scarce or lacking in syconia of the mammoni and mamme crops. In some closely related species (*Ficus pseudocarica* Miq., and *F. palmata* Forsk.) the syconia of all three crops contain staminate flowers. The fig is protogynous, the anthers maturing from six to eight weeks after the stigmas of the pistillate flowers in the same syconium are receptive.

The Smyrna Type.—Syconia of the Smyrna type mature after the pollination of their long-styled flowers and a resultant development of the ovaries into achenes. Without such stimuli the immature figs of both the breba and main crops usually shrivel and drop when about an inch in diameter. Sometimes a few brebas develop parthenocarpically. The embryo and the endosperm of mature achenes account for the excellent quality of Smyrna figs. Horticultural varieties belonging to this type include Lob Injir (Calimyrna), Kassaba, and Bardajic. Smyrna-type figs are grown commercially in Turkey, Greece, Algeria, Portugal, California, and to a small extent in Spain.

The White San Pedro Type.—Figs of the White San Pedro type bearing long-styled flowers combine the characteristics of both the Smyrna and the common type on one tree. Brebas are of the common type and have a parthenocarpic development. Second-crop figs are of the Smyrna type, the syconia dropping unless the flowers are stimulated by pollination and the ensuing fertilization of the egg cell. Examples of varieties belonging to this type are the White

San Pedro and the Gentile, neither of which is grown commercially to any extent.

The Common Type.—Figs of the common type have long-styled flowers which develop parthenocarpically and do not require the stimulus of pollination in order to have the syconia mature. Varieties of this type are the Mission of California, the Dottato of Italy (Kadota of California), and the Pajajero of Spain. The flowers of common-type figs were once regarded as incapable of producing fertile achenes and were therefore designated by Eisen (1896) as "mule flowers". Rixford (1918) and others, however, have proved that probably all common-type figs can produce fertile achenes if the flowers have been pollinated and fertilization has taken place.

Morphologically the flowers borne by figs of the Smyrna type and of the common type appear to be very similar. The difference between the two types lies in the fact that Smyrna fig receptacles, like the fruits of most angiosperms, require floral stimulation by pollination and the fertilization of the egg cell in order to develop to maturity.

PHYLOGENY OF THE FIG

Teratological specimens of figs described by Penzig (1894, p. 295) assist in explaining the structure of the syconium as a shortened, fleshy branch. This is especially noticeable when scales which normally line the ostiolum and sheathe the base or neck of the syconium appear on the surface in a regular, spiral sequence. Cook (1922) interprets these teratological forms as follows: "Since each of the scales may be supposed to represent the leaf of a specialized joint or internode of the fruit branch, the scales serve to indicate the number and arrangement of the internodes of which the fruit is composed. Interpreted in this way, the fleshy wall of the fruit evidently represents a series of internode elements standing side by side and completely fused though the scales remain distinct."

MATERIAL AND METHODS

Syconia of the various series of fruit buds were collected during the 1929 and 1930 season at the Citrus Experiment Station, Riverside; at the University Farm, Davis; and from an orchard near Fresno. In certain series of collections regular fixations of material were made from the first appearance of the fruit until the syconia were mature. Other series of collections were of shorter duration and made to show certain stages of development.

The material collected and studied during the two years represented three species of *Ficus* (*F. carica* L., *F. pseudocarica* Miq., and *F. palmata* Forsk.) growing in commercial orchards. Among these there were three horticultural varieties of the Smyrna type of fig, ten of the common type, ten of the caprifig type, three of the White San Pedro type, and two aberrant forms, the so-called Cordelia and the Hamma, which are edible caprifigs having both short-styled and staminate flowers in the same receptacle. Hamma figs require caprification in order to have the fruit set and mature but Cordelia figs develop by parthenocarpy.

A chrom-acetic-formalin killing and fixing solution of the following formula was used:

Solution A	*Solution B*
65 cc water	40 cc formalin (commercial)
10 cc acetic acid	35 cc water
1 gram chromic acid	

One part of solution A is mixed with one part of B just before fixing. This solution proved more satisfactory than Bouin's, Carnoy's or Carnoy's plus Karpechenko's in a comparative test. It evidently penetrated the partly sclerified ovary wall with sufficient rapidity to fix mitotic figures in the endosperm, although Tischler (1912) found it desirable to remove the sclerified wall of the ovary before fixing.

Small pieces of the syconium with flowers attached were put through the usual processes of killing and fixing, dehydrating, and embedding in paraffine. Individual flowers of later stages were also used. Sections were usually cut 10 microns in thickness and stained in Haidenhain's iron-alum hematoxylin. In order to obtain preparations showing certain desired stages of development, it was necessary to mount, stain, and examine hundreds of slides, a large percentage of which were afterwards discarded.

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STAMINATE FLOWERS AND THE DEVELOPMENT OF THE MICROGAMETOPHYTE

Mature staminate flowers normally consist of a slender pedicel bearing a five-parted perianth and five very short filaments each supporting an introrse two-lobed anther.

The early development of the male flower within the syconium is very similar to that of many other angiosperms. When first recognizable the primordium is a small knob of embryonic tissue (fig. 1 *C*), the apex of which generally develops into a rudimentary pistil having at its base the primordia of the anthers (fig. 1 *D, E*).

Previous to this investigation rudimentary pistils were thought to develop in occasional staminate flowers only (Longo, 1919; Ravasini, 1911; Cotte and Reynier, 1923), but the present study has shown that rudimentary pistils are normal and not exceptional in staminate flowers. None of the ovules, however, developed to the macrospore mother-cell stage.

Anthers of caprifigs develop rather slowly. For a considerable period after oviposition by the female blastophaga in pistillate flowers in the same receptacle, they consist of a more or less homogeneous mass of parenchymatous cells enclosed by an epidermis. At an early stage the young anther is cordate in cross section and later develops two microsporanges in each lobe. The sporogenous cells are distinguishable by their denser cytoplasm. The tapetal layer surrounding the sporogenous tissue is one or two cells in thickness and distinguishable by the smaller size of its cells and their smaller nucleoli. The epidermis consists of a single layer of small cells each of which has a thick outer wall, a large vacuole, and a nucleus embedded in cytoplasm adhering to the inner wall.

Bordering the epidermis on the inside is the endothecium (fig. 1 *F*), a prominent single layer of radially stretched cells with large vacuoles and lateral nuclei. On the thinner walls of the anthers the small epidermal cells are missing. The cells between the endothecium and the tapetum are parenchymatous and vacuolate with small nucleoli.

The sporogenous tissue consists of a mass of angular, large-nucleate cells extending almost the length of the anther, and is eight cells in diameter. The pollen mother cells, the last cell generation of this tissue, are at first angular; later they separate from one another, become rounded, and have somewhat thickened walls. During the

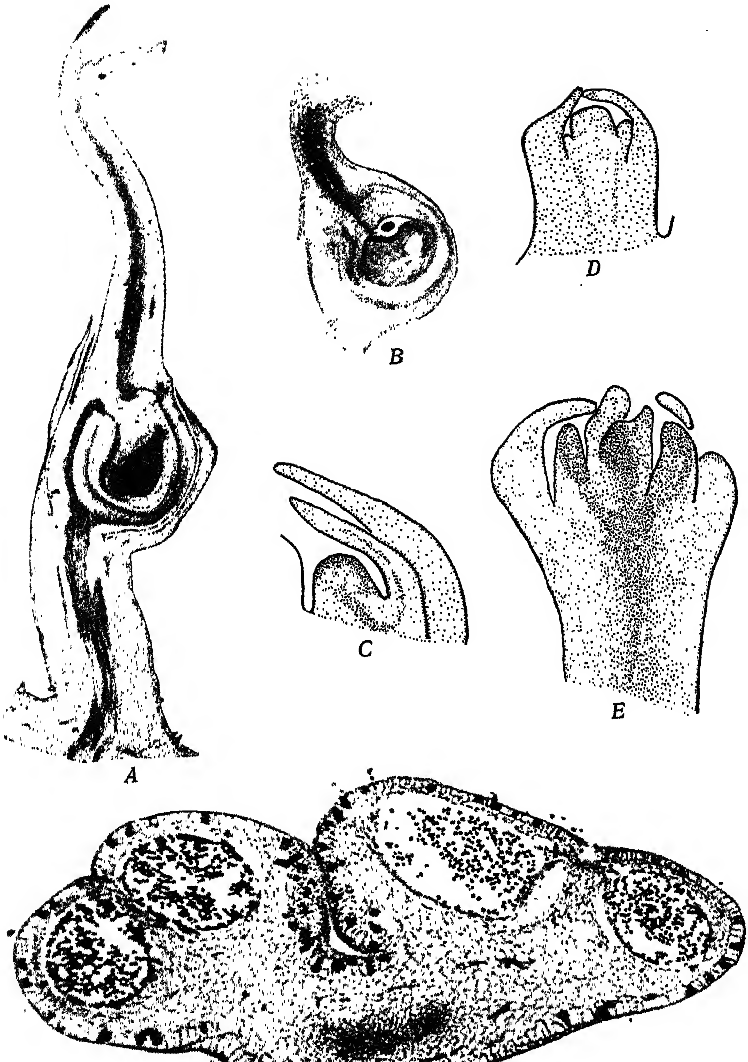

Fig. 1. *A*, A typical long-styled flower showing ovary, style, and two-lobed stigma. (X 28.)

B, A typical short-styled flower showing cells of stylar canal injured by ovipositor; the egg of blastophaga; the ovule and the embryo sac. (X 28.)

C, A staminate primordium with an over-arching ostiolar bract. (X 71.)

D, A staminate primordium with the anthers differentiating at its base. (X 71.)

E, A young staminate flower with anthers and rudimentary pistil. (X 71.)

F, Mature anther with four locules containing microspores, each locule bordered by an endothecium. (X 57.)

development of the pollen mother cells the tapetal layers gradually disintegrate and the cytoplasm of the intervening layers of cells practically disappears.

The heterotypic and homotypic divisions in the pollen mother cells follow one another in rapid succession. Both divisions may be going on simultaneously in the same anther. At that time well-developed tetrads may be present in neighboring anthers. As a result of the first division of the mother cells the number of somatic chromosomes (twenty-six) is reduced to thirteen (fig. 8 *A*) (Condit, 1928).

Microspores are usually tetrahedral in their arrangement in the tetrad stage, quadripartition taking place by furrowing. The production of microspores is profuse, several hundred being found scattered in each locule (fig. 1 *E*). Very few defective microspores are found, practically all showing cytoplasm and a nucleus.

The mature pollen grains are spherical or broadly ellipsoidal, without surface markings, and about 11 microns in diameter. Pollen grains "beautifully and characteristically sculptured" (Eisen, 1896) have not been found during the present investigation. Ellipsoidal pollen grains commonly have a germ pore at each pole; spherical grains usually have three pores. The pollen grains germinate readily in a 5 per cent sugar solution.

Cotte and Reynier (1923) observed shriveling of pollen grains of syconia in which the gall flowers fail to develop. Such is not the case in California, where normal pollen is produced in profusion in 'polleniferous' or 'blank' caprifigs destitute of insect galls. Longo (1909) also reports the maturing of such figs containing normal pollen.

In order to have material available for the study of pollen-tube development at various stages, a large number of syconia of two horticultural varieties of Smyrna figs were pollinated by blowing pollen through the ostiolum into the interior of each syconium by means of a small pipette. Collections of these artificially pollinated figs were made at frequent intervals during the first few days and at greater intervals later.

Pollination of the stigmatic surface is evidently followed almost immediately by germination of the microspores. Short pollen tubes were distinguishable on stigmas collected 1½ hours after pollination. After 4 to 6 hours the pollen tubes had penetrated into the stigma to a depth of several cells, the course of a single tube being traceable with difficulty and for a short distance only. Microtome sections stained in Haidenhain's iron-alum hematoxylin or in magenta red failed to reveal the course of the pollen tube through the style and into the ovule. Borthwick (1931) found resorcin blue very satisfactory in dif-

ferentiating pollen tubes and their callose plugs in preserved flowers of *Daucus carota* L. This stain, however, did not give similar results when used on preserved fig flowers.

During the course of his investigation of parthenogenesis in *Ficus hirta* Vahl., Treub (1902) observed pollen grains germinating on the stigmatic surface but failed to find any evidence of pollen tubes either in the style or ovule, even in flowers where there was a development of the embryo. Longo (1909), who holds that the fig ovule lacks a micropyle, states that the slender pollen tube goes through the central collenchymatous tissue of the style, then through the apex of the internal integument to the apical region of the nucellus where the cells are especially rich in cytoplasm. He gives a diagrammatic illustration of the course of the pollen tube.

The growth of the pollen tube through the style and into the ovule is evidently fairly rapid. At Riverside, California, numerous syconia of Calimyrna fig trees were caprified on July 19. Flowers of syconia collected on July 23 showed a considerable development of endosperm and two days later several preparations of ovules showed two-celled embryos.

The stigmatic surface of short-styled caprifig flowers is just as favorable for the germination of pollen as that of long-styled flowers found in other types of fig. There is commonly a pollination of flowers of the mammoni and mamme crops, and fertile achenes are frequently found among the gall flowers in mature caprifig syconia. Leclerc du Sablon (1908*b*) thinks that mammoni and mamme flowers normally have pollination and fertilization but that the development of the embryo is stopped by the growing larva. However, no investigator has ever discovered a gall flower containing both an immature embryo and a young blastophaga larva.

The reason for the absence of an embryo in flowers containing a blastophaga larva becomes evident when one examines the style of these flowers. The cells of the stigmatic surface are more or less injured as the insect seeks to insert its ovipositor into the funnel-shaped apex of the style. There is also serious injury (fig. 1*B*) to the cells lining the stylar canal by the insertion and withdrawal of the ovipositor. Thus, even though pollen grains germinate, the growth of pollen tubes between the injured cells appears to be absolutely inhibited. The embryo of a plantlet and the larva of a blastophaga therefore could not be found in the same gall flower unless the embryo should develop parthenogenetically, or as the result of pollen-tube growth and fertilization of the egg before oviposition by the blastophaga.

PISTILLATE FLOWERS AND THE DEVELOPMENT OF THE MACROGAMETOPHYTE

Mature pistillate flowers within a syconium typically have the following parts: a pedicel, five perianth lobes partly enclosing the spherical ovary, a simple style, and a stigma usually cleft into either equal or unequal limbs.

The primordia of pistillate flowers are at first indistinguishable from the primordia of staminate flowers. Normally in the primordia of both types of flowers the embryonic mass soon forms a symmetrical, encircling collar just below its apex. This collar becomes asymmetrical by its unequal development (fig. 2*B*). In pistillate primordia one side of the collar elongates more rapidly than the other, and eventually develops into the long limb of the stigma (figs. 2 *C, D*). The other side of the collar, which grows more slowly, develops into the short stigmatic limb. A central pore or stylar canal (fig. 2 *D*) more distinct in some styles than in others, is left as the collar elongates. No long-styled flowers with staminate primordia have been found during this investigation, although Leclerc du Sablon (1908*a*) reports the occurrence of such hermaphroditic flowers in rudimentary stages.

The primordia of both staminate and pistillate flowers are borne on pedicels of various lengths, the surface of which may be smooth or armed with scattered spicules. One-celled spicules similar to those on the outer epidermis of the syconium are thickly scattered on the inner surface of the receptacle between the pedicels.

Perianth.—The primordia of the perianth lobes (fig. 2 *A*) appear in acropetalous succession at the base of the central pistillate primordium before the formation of the apical collar. The fig flower, typically pentamerous, may have only three or four perianth lobes or occasionally six or seven. According to Eisen (1896) the "petals" of both staminate and pistillate flowers are generally four in number but those of staminate flowers are shorter than those of pistillate flowers. Leclerc du Sablon (1908*a*) calls the perianth lobes "*sepales*" and states that their number, though generally four or five, is of no more importance than the number of stamens, usually four or five. In this investigation actual counts of the perianth lobes of 71 flowers from six horticultural varieties of figs and caprifigs showed 31 flowers with five lobes, 19 with four, 16 with six, 4 with three, and 1 with seven lobes.

The perianth lobes are mostly broadly lanceolate and united to one another at the base. Occasionally two lobes are joined together for half their length or more. The margins of the perianth lobes are mostly entire, though sometimes serrate near the apex, and are generally armed with prominent apical spines or spicules. The lobes

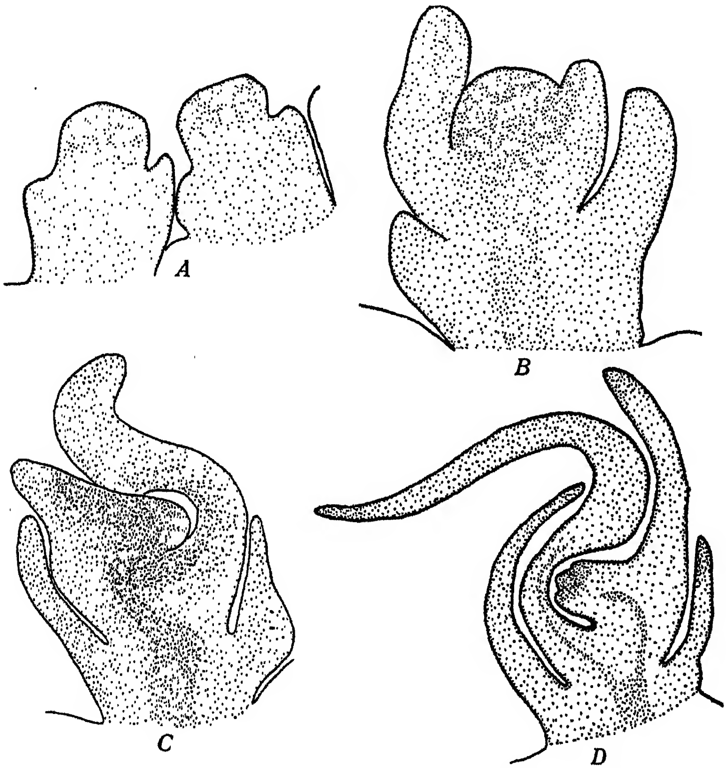

Fig. 2. *A*, Perianth lobes differentiating laterally from primordia of pistillate flowers. (X 184.)
B, Primordium of pistillate flower differentiating at its apex into an asymmetrical circular collar. (X 184.)
C, Further differentiation of apex of primordium into style and stigma. (X 133.)
D, Long-styled flower after differentiation from the primordium of ovary, style, stylar canal, unequal stigmatic lobes, and perianth. (X 75.)

may be equal in length to the style in short-styled flowers but are always much shorter than the style of long-styled flowers.

Style.—The stylar primordia, formed in the manner already described, develop into short, stubby styles (fig. 1 *B*) characteristic of flowers borne in syconia of the caprifig type or into long, slender

styles (fig. 1 *A*) found in flowers of the Smyrna, White San Pedro, and common types of figs. In general, the length of style in short-styled flowers is from 0.55 to 0.70 mm and in long-styled flowers from 1.5 to 2.0 mm. The thickness, form (straight, curved, or bent at an angle), and length of style in long-styled flowers vary considerably according to the horticultural variety of fig studied. These and other characters are of taxonomic value in the study of fig varieties. Leclerc du Sablon (1908*a*) found a continuous series of flowers ranging in length from those with short styles to those with very long styles.

The surface of the style of fig flowers may be smooth or studded more or less thickly with sharp, slender spicules. Within some of the cells of the style there are commonly found single, large crystals of calcium oxalate, as mentioned by Ravasini (1911).

Various writers refer to the style of caprifig flowers as being hollow, especially toward the apex, although both Longo (1909) and Ravasini (1911) describe it as funnel-shaped, a better descriptive term. The funnel-shaped apex of the style assists the blastophaga in introducing her ovipositor into the stylar canal.

Stigma.—As already stated, bifid stigmas (fig. 1 *A*) are characteristic of most fig flowers. This character, however, is somewhat variable, both simple and bifid stigmas being found in flowers of the same receptacle. Flowers of the Mission fig may have simple stigmas or there may be found a second very short stigmatic lobe at the base of the longer one. In most bifid stigmas one lobe is much longer than the other. The stigmatic lobes of many varieties of common and of Smyrna-type figs are very prominent, almost equal in length, and spread out horizontally. The lobes of the stigma of the Hamma fig are unusually long, much longer in fact than those of any other fig flower examined. Actual measurements of the stigmatic lobes of various fig flowers show the following:

Variety	Length of longer limb *mm*	Length of shorter limb *mm*	Length of simple stigma *mm*
Calimyrna	0.77 to 1.08	0.40 to 0.66	....
Mission	.88	.16 to .33	1.00 to 1.10
White San Pedro	.88 to .99	.35 to .75	....
Hamma	.99 to 1.26	.66 to .93	....
Roeding No. 3 caprifig	0.40 to 0.70	0.10 to 0.40	0.50 to 0.55

Flowers of brebas and second-crop figs of the same variety are similar in stigmatic characters.

The line of demarcation between the surface of the style and stigma is often difficult to determine when entire flowers are examined.

In longitudinal sections, however, the demarcation is usually apparent. The epidermal cells in the region of the stigma are rectangular with thick outer walls. The stigmatic surface consists of elongated, diagonally arranged cells with thin walls. These papillate cells are found on the stigmas of both long and short-styled flowers.

The stigmas remain receptive to pollen two weeks or more at ordinary temperatures.

Ovary.—The ovary is formed from the basal part of the immature pistil. As seen in longitudinal section the ovary is asymmetrical, the wall on the side of the funiculus and the short limb of the stigma being much thicker than the other. The stylar canal leads to the locule of the ovary which is almost completely filled by the ovule.

The ovary wall consists of more or less distinct layers (four according to Ravasini, 1911) of cells (fig. 3). At the outside is an epidermal layer continuous with and similar to that of the style. The cells of the innermost layer are continuous with but larger and more regular in shape than those lining the stylar canal. During the period of development of the macrogametophyte their general appearance is similar to that of the epidermal cells. Between these two layers are three or four other tiers of cells showing at first no special differentiation. At the macrospore mother-cell stage the spiral vessels of the vascular tissue are distinguishable in the intermediate cell layers of both the thick and thin portions of the ovary wall. Adjacent to the innermost layer there becomes differentiated a single or sometimes double layer of small cubical or palisade-like cells (fig. 3) rich in cytoplasm. Further differentiation of the cells composing the various layers takes place after maturity of the macrogametophyte.

Oviposition by the blastophaga in the short-styled flowers and the development of the young larva result in a gradual thickening of outer walls in the epidermal layer. There is also a gradual sclerification of the two inner layers of cells forming the ovary wall. Sclerotic cells are well developed and section with some difficulty even while the larva is still fairly young. Material showing eggs of the blastophaga on April 3 showed young larvae and well-sclerified ovary walls on April 21. The greatest amount of sclerification takes place in the cells of the innermost layer, the adjacent small square cells becoming sclerified but remaining about the same size. The epidermal and intermediate cell layers remain attached as a dry outer covering to the sclerified ovary wall of the mature gall flower. According to Leclerc du Sablon (1908*b*) there is in the same syconium less sclerification in the ovary wall of flowers which harbor a blastophaga larva than in the wall of the few flowers which contain a plant embryo.

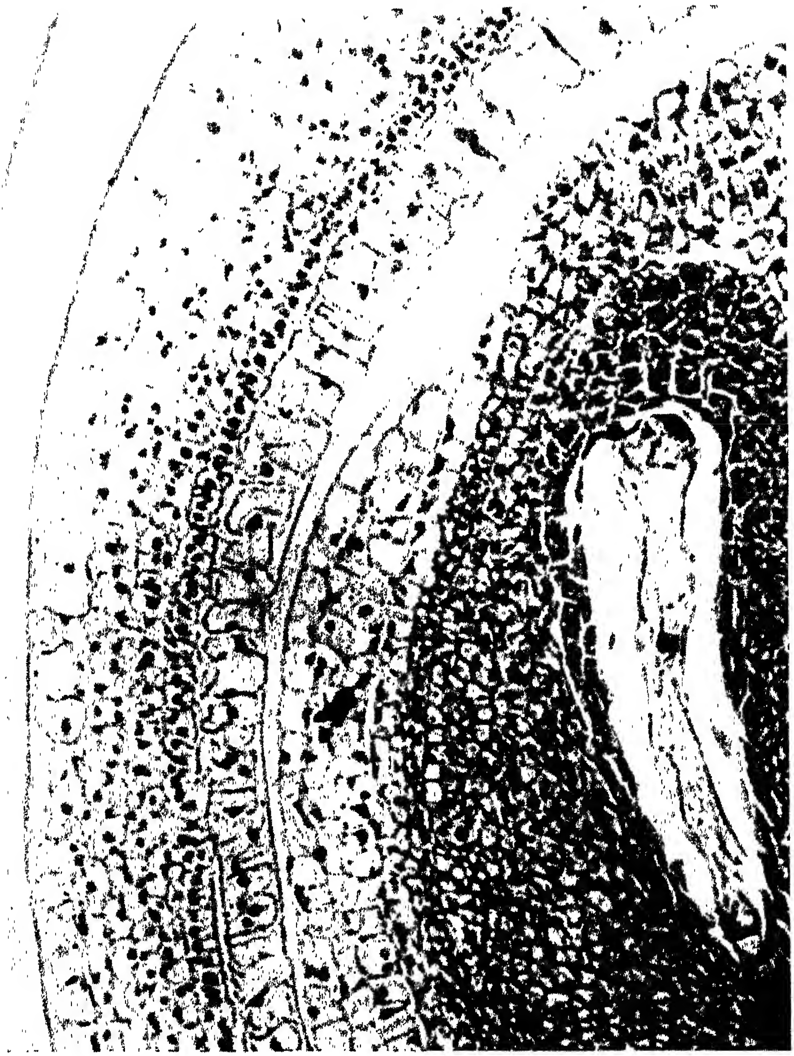

Fig. 3.—The ovary at the time of maturity of the macrogametophyte, showing the cell layers of the ovary wall, the outer and inner integuments, and the nucellus enclosing the embryo sac. (X 450.)

There is, however, but little sclerification at the junction of the funiculus and the base of the style. The adult blastophagas escape through this partly sclerified region in the ovary wall. Short-styled flowers in which there is neither a blastophaga nor an embryo do not, with the exception of such figs as the Cordelia, have any development or sclerification of the ovary wall. The achenes of long-styled flowers, which never contain a blastophaga, usually have a well-sclerified ovary wall.

Cell layers in the young ovary wall of long-styled flowers are very similar to those of the short-styled flowers. Sclerification (fig. 4) takes place in the same way and to about the same extent in the innermost cell layer and in the adjacent small cells. The epidermal cells become considerably enlarged and radially stretched and their outer walls thicken materially (fig. 7). The pulpy part of edible figs consists of the thicker layers of unsclerified cells in the ovary wall. These cells, as well as those of the pedicel and style, remain parenchymatous and serve as storage tissue. Some caprifigs have a certain amount of such parenchymatous tissue which becomes pulpy. In the Cordelia fig the surface cells of the ovary wall are developed to the same extent as in most edible figs with long-styled flowers. Adult blastophagas if present in the mature Cordelia figs usually perish in the mass of pulpy flowers. Pulpy, edible caprifigs are also common in the mammoni crop of such varieties as the Milco.

Such horticultural varieties of the fig as Mission, Brown Turkey, and White Marseilles have numerous hollow and infertile achenes with the ovary wall fully sclerified. Other varieties with infertile achenes, such as the Dottato (Kadota) and Brunswick (Magnolia), do not have the ovary wall as fully sclerified nor as well developed as in the plump achenes of most common figs. To call such figs seedless is incorrect; Traub and Fraps (1928) record an average of 406 infertile achenes in Magnolia figs.

The term *phenospermy* is used (Goodspeed, 1915) to describe the production of seeds which appear to be normal but consist of empty seedcoats only. The term *cenocarpy* is hereby proposed to describe the production of a fruit or an achene which has a normally developed ovary wall but does not contain an embryo.

The stone cells of the sclerenchyma in the ovary wall of fig flowers are sufficiently characteristic of the species to enable their identification in food preparations such as marmalade, jam, and coffee substitutes. According to Winton (1916, pp. 386–390), who reproduces the account and illustrations of Moeller (1886, pp. 287–290), the outer sclerenchyma consists of a single layer of small stone cells 15

Fig. 4.—Immature achene showing *a*, the sclerified ovary wall; *b*, the seed coat formed by the integuments; *c*, the endosperm cells, cambium-like in appearance; and *d*, portion of the young embryo. (X 450.)

microns in diameter. The endocarp or inner sclerencyma is composed of one or more layers of rounded, angular stone cells about 50 microns in diameter. Each cell has a narrow lumen and thick walls with distinct, concentric layers perforated by branching pores.

Ovule.—There is usually a single ovule within the ovary. Bi- or triovulate ovaries with more or less deformed ovules are more frequent in some horticultural varieties, such as the White San Pedro, than in others. Longo (1909) states that in first-crop figs multiovulate ovaries are more common than uniovulate ones.

Specimens of the Black Douro variety of fig collected at Fresno, April 16, showed two distinct types of flowers with respect to the ovary, one with a normal and the other with a double ovary. Such flowers were also found, but less commonly, in the Dauphine, Datte, and Black Ischia varieties of fig. Flowers with a double ovary are symmetrical, with equal bulging sides and similar stigmatic lobes. Sections show a central vascular strand in the pedicel branching off on either side to the funiculus of each ovule. However, no mature achenes with a double ovary have been found. Similar biovulate ovaries have been recorded by Solms-Laubach (1882) and by one or two other writers.

The ovule and the stigmatic lobes differentiate about the same time. The very young ovule is a hemispherical meristematic mass (the nucellus), from the base of which there successively develop the inner and then the outer integuments, the inner growing more rapidly. At the time of differentiation of the macrospore mother cell, the apex of the nucellus is in some flowers almost completely enclosed by the inner integument. In other flowers the nucellus is only half covered by the inner integument at this stage. At the time of maturity of the macrogametophyte, a cap-like mass of cells of the inner integument covers the micropylar end of the nucellus (figs. 3, 5) about as deeply as the underlying nucellar cells cover the cavity of the sac itself. Longo (1909) states that in multiovulate ovaries the nucellus is not always covered by the internal integument but protrudes from it like a large, more or less pointed nipple. The outer integument (fig. 3) never develops sufficiently to cover the apical portion of the inner integument.

Leclerc du Sablon (1908 *b*) states that each integument is five cells in thickness, the cells of the outer integument generally persisting during development of the ovule, those of the inner one being digested by the underlying tissue. My preparations show the inner integument to be three or more cells in thickness (fig. 3), the internal layer consisting of rectangular cells much smaller than the nucellar cells

Fig. 5.—Mature macrogametophyte with synergids, egg cell, two polar nuclei, and two of the three antipodals. A micropyle is plainly in evidence at the apex of the inner integument. (X 450.)

adjoining. The external layer is composed of large, more or less oblong cells with the nucleus and cytoplasm next to the outside wall. The outer integument is also from three to five cells in thickness (fig. 3), the individual cells being still larger than the adjoining cells of the inner integument.

Fig. 6.—Mature achene with embryo surrounded by endosperm, seed coat, and pieces of sclerified ovary wall. (X 68.)

In long-styled flowers the internal layer of the inner integument and the external layer of the outer integument become filled with a deeply stainable substance (figs. 4, 7) during the early stages of embryonal development and also during the development of cenocarpic achenes. These prominently staining layers persist unchanged in the seed coat developing from the integuments (fig. 6) but the other integumentary cells lose their contents and become more or less crushed during the later stages of achene development.

Short-styled flowers containing the larva of a blastophaga do not have this differentiation of the integumentary layers, their cells being finally consumed by the developing larva. Achenes of short-styled flowers in which there is development of an embryo, do show a differentiation of the integuments into a seed coat.

A micropyle is distinctly in evidence at the two-nucleate stage of the macrogametophyte. A clearly defined micropylar canal can rarely be found at the time of maturity of the macrogametophyte, the opening being obliterated by the closing together of adjacent cells of the inner integument. There must, however, be a line of demarcation between the cells. The presence of a micropylar canal can occasionally be demonstrated, as in the ovule shown in figure 5.

The presence or absence of a micropyle was the main basis for a discussion between Ravasini (1911, 1912*a*, 1912*b*), who described and figured one, and Longo (1911*a*, 1911*b*, 1912*a*, 1912*b*) who contended it was lacking at the two-nucleate (or at most four-nucleate) stage of the young embryo sac. Tischler (1912) also reported the absence of a micropylar opening at the completion of the eight-nucleate stage of the embryo sac.

The nucellus itself is composed of an immense number of undifferentiated cells irregularly arranged except at the micropylar end, where they are in more or less vertical rows. Some ovaries show cases of nucellar budding or continued meristematic activity in various parts of the nucellus, resulting in odd proliferations or even in secondary primordia with a macrospore mother cell.

The development of the macrosporange and of the macrogametophyte of fig flowers is similar to that occuring in many angiosperms. There are no perceptible differences in the macrogametophytes of short-styled and long-styled flowers. Both kinds of flowers are truly pistillate and usually develop with an eight-nucleate macrogametophyte. Furthermore, the present evidence indicates that the embryo sacs in first and second-crop figs and in the flowers of all crops of the caprifig tree are similar, all embryo sacs containing an egg cell and a polar nucleus being potentially capable of fertilization.

In the development of the ovule the macrospore mother cell becomes deeply embedded within the nucellus, because of an extensive development of the parietal tissue, the cells of which are regularly arranged. The reduction division was observed in a few cases but chromosome counts were not obtained. Some preparations showed the linear tetrad or the four-macrospore stage but none were found showing clearly the nonfunctional spores or their degeneration. The tetrads are

not always in a linear arrangement, the four macrospores sometimes being found in two adjacent groups near the center of the sac.

After the division of the nucleus in the functional macrospore the daughter nuclei migrate, one to each end of the embryo sac. The four-nucleate and the eight-nucleate stages soon follow, the latter being attained while the embryo sac is still much smaller in size than it is at maturity. In the mature macrogametophyte, the egg and two synergids are at the micropylar end, the two polar nuclei at the center and the three antipodals at the chalazal end (fig. 5). The egg is indistinguishable in size and appearance from the synergids. The fusion of the polar nuclei takes place well before the time of fertilization.

An ovule with a complete embryo sac is shown diagrammatically by Ravasini (1911). Leclerc du Sablon's (1908*b*) preparations showed mature embryo sacs but not the egg apparatus or the antipodals. The synergids were found by Tischler (1912) to be similar to the egg cell, differing only in their much smaller protoplasmic content. He illustrated the egg and synergids but not the antipodals, which were poorly differentiated in his material.

THE ENDOSPERM

The Development of Normal Endosperm.—There is no reason for believing that development of endosperm in the fig flower is other than normal, even though the actual fusion of a sperm nucleus with the polar nuclei has not been observed. This is indicated by the fact that mitoses of endosperm nuclei show approximately the 3n complement of chromosomes (fig. 8 *B*). There is a considerable multiplication of endosperm nuclei before the division of the zygote. A continuous series of thirteen sections of one ovule, with the embryo at the two-celled stage, showed 273 free endosperm nuclei well distributed about the periphery of the embryo sac. Chromosome counts are not at all difficult in somatic mitoses of properly prepared root-tip material of the fig; polar views of metaphase plates show the chromosome complement well distributed and mostly in one plane (Condit, 1928). But chromosome counts of metaphase plates of endosperm nuclei are made with difficulty, not so much on account of the extremely small size of individual chromosomes as on account of their close grouping in a dense mass of cytoplasm. Counts of a few metaphase plates (fig. 8 *B*) showed from 33 to 37 of the 39 chromosomes which should be present.

The nuclei of the young endosperm are spherical or ellipsoidal in shape and 6.5 to 10.0 microns in diameter. Both the nuclei and nucleoli are at least twice the size of those in the adjacent nucellar and integumentary tissue. A single nucleus may have one large nucleolus or as many as five nucleoli equal or unequal in size. The peripheral zone of free-nucleate endosperm becomes cellular by cleavage when the spherical embryo consists of three or four hundred cells, the delicate plasma membranes appearing first in the region of the embryo.

The newly formed cells are at first polygonal but later those toward the periphery enlarge radially and have a more or less regular arrangement of their plasma membranes. The further development of this tissue must be interpreted from the arrangement of cells since divisions in cellular endosperm were not found in my material. The central cavity of the embryo sac becomes completely filled with uninucleate protoplasts, and these irregular protoplasts at the center eventually become separated from one another by cellulose walls. The cells at the periphery apparently divide periclinally, forming a layer of small cells regularly arranged and cambium-like in appearance (fig. 4). This process of cell formation by cleavage in the normal endosperm is quite different from the furrowing process described later in the parthenogenetic development of endosperm in short-styled flowers.

Nuclear fusions may sometimes occur during the early cellular stages in development of the normal endosperm, but to a very much smaller extent than in a parthenogenetic endosperm. As Leclerc du Sablon (1908*b*) has shown, albuminoid granules appear in the cellular endosperm after the cotyledons of the embryo have become well differentiated. He holds that many of these granules are of the nature of aleurone grains. In the mature achene (fig. 6) the endosperm cells are densely packed with spherical granules of different sizes. According to Winton (1916, p. 389) the mature endosperm makes up about half of the bulk of the achene and consists of thick-walled, polygonal cells containing proteid matter and fat.

The Parthenogenetic Development of Endosperm.—The flowers of any type of fig which have had a normal development of the macrogametophyte and into which no sperm nuclei have entered, may have a development of endosperm. Such endosperms are called parthenogenetic but their development is not the same in long and in short-styled fig flowers.

The most careful cytological study of this parthenogenetic development of endosperm has been made by Tischler (1912) with material

from the syconia of a common-type fig containing long-styled flowers. He finds that the first cells formed in the endosperm are often multinucleate but that later many become uninucleate by a fusion of their nuclei. The process of cell formation is not described, although he states it proceeds independently at opposite poles of the embryo sac.

Tischler describes the endosperm nuclei as being very irregular in size owing to their fusion, a phenomenon seen in all stages, especially in the multinucleate cells. The differences in size of nuclei and cells are found to be more striking in the mature endosperm than in the early stages. Tischler failed to find mitoses of endosperm nuclei but does not conclude, as does Longo (1909), that division is by fragmentation. He states that the cells of the mature parthenogenetic endosperm contain considerable reserve material similar to that of the normal endosperm. Later he found a disintegration or digestion of the mature cells, taking place from the center towards the periphery, through the action of enzymes secreted by the endosperm cells themselves.

Tischler states that the egg cell usually persists, and in some cases increases to an immense size, greater than that reported for the egg cell of any other angiosperm. One of his preparations showed that there had been repeated mitoses of the egg nucleus but this was not followed by cytokinesis. This egg cell contained approximately 132 free nuclei.

During the present investigation, a certain amount of parthenogenetic development of endosperm was found in long-styled flowers collected from brebas of five varieties of the common type, two varieties of the White San Pedro type, and one of the Smyrna type of fig. Since collections of material were not made for the express purpose of studying this parthenogenetic endosperm, a continuous series of developmental stages was not obtained. The first division of the nucleus resulting from the fusion of the two polar nuclei and subsequent divisions of the daughter nuclei proceed tardily. The rate of development is also irregular from flower to flower. Material collected on April 3 showed long-styled flowers with stigmas receptive to pollen and with fully developed embryo sacs. Material collected on April 29 from the same trees showed in many ovaries a single, prominent, polar fusion nucleus and a degenerated egg apparatus; but some ovaries showed a very scanty and others a profuse development of the endosperm (fig. 7). In a few cases the daughter nuclei were found to be densely clustered in small masses rather than widely distributed in a peripheral layer. Mitotic figures, though scarce, were normal. Counts of a few metaphase plates showed 24 or 25 chromosomes, indicating the 2n complement of chromosomes.

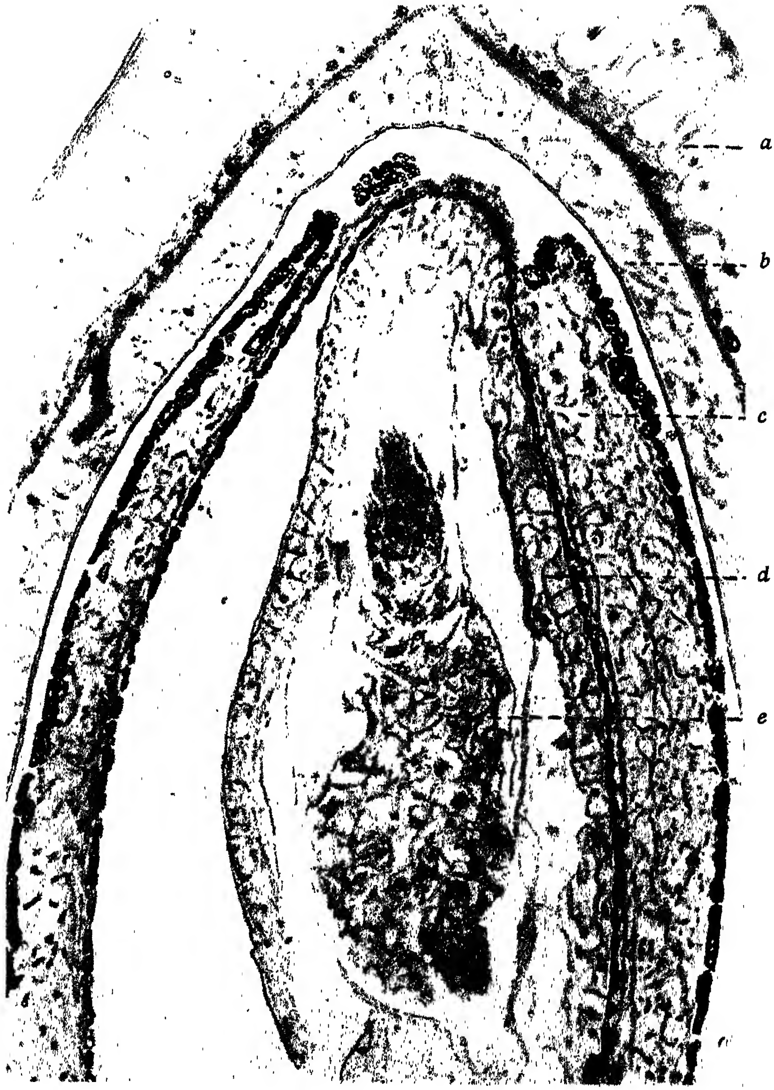

Fig. 7.—Immature ovary or achene of Mission fig flower, breba crop. *a*, Enlarged epidermal cells; *b*, inner cells of ovary wall becoming sclerified; *c*, remains of inner and outer integuments with prominently staining layers; *d*, remains of nucellus; *e*, parthenogenetic endosperm. (X 207.)

The nuclei and nucleoli are in general very similar in size and shape to those of the normal endosperm. Nuclear fusions were found in my preparations, but the absence of striking irregularities in form of nuclei and in aberrant mitotic figures indicate that they are not of common occurrence.

Cell formation seems to take place in the same manner as in the normal endosperm, but not enough stages were found to determine this with certainty.

As already noted (p. 453), oviposition by the blastophaga so injures the stylar canal that there cannot be a growth of pollen tubes to the ovule. However, such flowers regularly develop endosperm, a tissue which furnishes the main subsistence of the blastophaga larva. The obvious inference that this endosperm is parthenogenetic is confirmed by the determination of chromosome number as shown later.

The development of this type of endosperm has been studied by Longo (1906, 1909) and by Leclerc du Sablon (1907, 1908*b*). Both investigators state that development of this parthenogenetic endosperm is stimulated by deposition of the blastophaga egg in the ovule just as normal endosperm development is stimulated by the union of the sperm nucleus and the polar nuclei. The fact remains, however, that endosperm development sometimes takes place in the ovules of parthenocarpic figs without the stimulus resulting from oviposition by the blastophaga or from the entrance of a sperm nucleus. Such figs show that neither oviposition nor fertilization is essential in inducing development of endosperm. Coulter (1911) has pointed out that endosperm formation is not dependent upon the entrance of a male nucleus nor even upon the fusion of polar nuclei, and that both of these fusions may be regarded as supplementary rather than determinative. In his work on *Fritillaria*, Sax (1918) found that the development of endosperm was independent of the development of the embryo. He also found about a dozen cases in wheat where the endosperm developed normally with no embryo present.

The rate of division of the endosperm nucleus may be determined from the stage of development of the blastophaga. According to Grandi (1929) the egg stage of the blastophaga lasts 4 or 5 days. Several of my preparations show that two or three successive divisions of the endosperm nucleus took place while the blastophaga was still in the egg stage. In these flowers, therefore, development of the parthenogenetic endosperm must have followed closely after oviposition. Most flowers, however, do not have a development of the endosperm nucleus until the blastophaga has reached the early larval stage. Even then nuclear divisions proceed rather slowly.

During the early period of development of parthenogenetic endosperm in the fig ovule, the synergids and antipodals disintegrate. The egg cell may or may not persist. Some egg cells show a slight increase in size, but none show any indication of mitosis of the egg nucleus.

In my preparations the first few divisions of the endosperm nucleus result in nuclei very similar in size and shape to those of normal endosperm. The peripheral region of the parthenogenetic endosperm has a denser cytoplasm than that of the normal endosperm and the outlines of the nuclei are not so clearly defined. The individual nuclei are spherical, ellipsoidal, pyriform, or elongated in shape.

Fig. 8. *A*, Two haploid sets of chromosomes from metaphase plates of homotypic division in the pollen mother cell. (X 2,600.)
B, A metaphase plate of mitosis of a normal endosperm nucleus with 37 of the 3n complement of chromosomes. (X 2,600.)
C, A metaphase plate of mitosis of a parthenogenetic endosperm nucleus with the 2n complement of chromosomes. (X 2,600.)

Mitoses of nuclei in the free-nucleate stage of the parthenogenetic endosperm have not been found by previous investigators, but mitotic figures in all stages have been found in abundance during the course of the present investigation. Divisions of the free nuclei are not simultaneous, resting nuclei and mitotic figures being found side by side. Here again exact chromosome counts are difficult to make in metaphase plates since the chromosomes are not spread out in a single plane as they are in somatic divisions. Several counts, however, showed clearly that in the free-nucleate stage the chromosome complement is 2n (fig. 8 *C*).

Cell formation, according to Leclerc du Sablon (1908 *b*) starts among the exterior nuclei but does not continue throughout the mass of parthenogenetic endosperm, a zone of free nuclei always being distinguishable toward the center. He does not explain the process by which cells are formed.

There are two general methods in angiosperms by which a multinucleate endosperm becomes a cellular tissue. Cell formation by means of cell plates was described by Strasburger (1880), and his illustration has become widely known by its publication in various

editions of the "Bonn" textbook (Strasburger, 1895). The cell plates are formed on parallel groups of kinoplasmic threads connecting the free nuclei. Such threads, while similar to, are not identical with the spindle fibers found in mitotic figures; they are special fibers concerned solely with cytokinesis. The cell plates of these fibers divide in the same manner as the cell plates of mitotic spindles and cell walls are then secreted between the membranes separating the naked protoplasts.

Cell formation may also be brought about by a progressive cleavage similar to that found in certain multinucleate fungi and algae (Harper, 1899, 1900; Timberlake, 1902). Frye's (1902) studies in certain Asclepiadaceae show that the multinucleate endosperm becomes cellular by progressive cleavage.

My preparations show that cell formation in the parthenogenetic endosperm of the caprifig is brought about by a more or less simultaneous process of cytoplasmic furrowing as in the second method mentioned above. This begins with the appearance of lighter-colored zones towards the exterior of the dense cytoplasm. There are no indentations at the periphery suggesting the inception of cleavage furrows in the plasma membrane. The lighter-colored zones in the cytoplasm broaden and there appears in the middle of each a distinct furrow (fig. 9 *A*). These furrows are very irregularly arranged with respect to one another. They gradually separate the cytoplasm into irregular blocks containing one or more nuclei. The furrows eventually extend to the surface of the multinucleate endosperm but the outer cells formed by furrowing are as irregular in size and shape as those toward the center. Nuclear divisions and furrowing continue until the central cavity is almost or completely filled with angular protoplasts. The blastophaga larva generally lies in a concavity (fig. 9 *B*) of this cellular endosperm but is occasionally completely surrounded by endosperm tissue.

The process of cell formation just described is analogous to the process of spore formation in certain fungi and algae. According to Swingle (1903), spore formation in *Phycomyces nitens* is brought about by the formation of angular vacuoles which by branching, curving, and intersecting, eventually form multinucleate bits of protoplasm. Czempyrek (1930) finds that swarm-spore formation in the multinucleate cells of *Cladophora* is brought about by vacuolation of the cytoplasm.

As already stated, nuclear fusions are found both in the normal and in the parthenogenetic endosperm of long-styled fig flowers, but are more frequently found among the endosperm nuclei of short-styled

Fig. 9.—*A*, Distinct furrows separating the cytoplasm into irregular blocks containing one or more nuclei, the larger ones polyploid. (X 450.) *B*, *a*, Blastophaga larva lying in a concavity of the cellular endosperm (*b*); *c*, remains of the nucellus; *d*, the partly sclerified ovary wall. (X 68.)

flowers. These fusions may occur during the free-nucleate stage but are more abundant after the formation of the multinucleate protoplasts. In fact, whenever two or more adjoining nuclei come into close contact, the individual membranes may disappear and the chromatin of the fusing nuclei become indistinguishable.

Nuclear fusion in multicellular endosperm was reported by Strasburger in 1880 for several species of plants, notably *Corydalis cava.* Tischler later (1900) examined other material of *Corydalis* and found that the two or more nuclei of a multinucleate cell often fuse, the subsequent divisions being irregular and the chromosome numbers exceedingly variable. The cases of nuclear fusion in endosperm cells have been listed by Tischler (1922, p. 507). In all of these, the resultant nuclei are extremely variable in size and shape.

Dixon (1895) decided that some of the polyploid nuclei in the endosperm of *Fritillaria imperialis* L. divide amitotically, the giant nucleus first assuming the shape of an hour-glass and then breaking into fairly equal portions. A decade later Saame (1906), studying the same species, interpreted such hour-glass-shaped nuclei as fusion stages. Schürhoff (1915) gives an excellent account of amitosis in the resting endosperm nuclei of *Ranunculus acer.* Schnarf (1929, p. 329) lists a number of plants in which there is amitosis during later stages of endosperm development, but he does not record whether these nuclei have the typical 3n complement of chromosomes or are polyploid. Longo (1909) found no mitoses in the nuclei of fig endosperm and so inferred that division was by fragmentation.

Polyploid endosperm nuclei have been shown to divide mitotically also. Soltwedel (1882) found both regular and irregular mitotic figures in several species of angiosperms with polyploid endosperm nuclei and thought further fusion took place during mitoses of neighboring nuclei. Both normal and abnormal mitotic figures as well as cases of amitosis, were observed by Dixon (1895) in polyploid nuclei of *Fritillaria.* Tripolar and quadripolar spindle figures were frequently found to form three or four daughter nuclei. Leclerc du Sablon (1908*b*) states that the mitotic figures in the parthenogenetic endosperm of the fig have an unusually large number of spindle fibers but the chromosomes are not clearly defined.

Some of the material obtained in this investigation, especially that of two forms of *Ficus palmata* Forsk., is unusually favorable for the study of mitotic figures in the polyploid nuclei of cellular endosperm. Metaphase plates or telophases in the division figures (fig. 10 *A*) of free nuclei are about 7.5 microns in diameter and have the 2n complement of chromosomes. Large metaphase plates (fig. 10 *B*),

11 microns in diameter, are found during mitoses of polyploid nuclei. These indicate a chromosome complement several times n, although the exact number could not be determined. Conversely, there were very small mitotic figures having metaphase plates much smaller than those of diploid nuclei.

Dixon (1895) found that in multipolar mitotic figures of endosperm nuclei one of the poles appeared to have less attraction for chromosomes than the others, the daughter nuclei being unequal in size. He also found cases in which a few chromosomes with spindle fibers became detached from the metaphase plate. In my preparations the polyploid nuclei of fig endosperm frequently show, during mitoses, an irregular migration of chromosomes toward the poles. Small groups of chromosomes may become detached from the larger group (fig. 10 *C*) and form part of the fused nucleus, or they may become separate nuclei that undergo further mitoses.

Abnormal and strikingly irregular spindle figures are characteristic of the parthenogenetic fig endosperm after it has become cellular. The abnormal figures vary considerably in the shape and arrangement of their spindles. Many of the spindle figures are very broad and have a width of 50–55 microns (fig. 10 *C*). Some of the transversely elongated figures are typical except for their unusual breadth. Others are more or less curved into very unusual and striking shapes. Some elongated figures have a homogeneous set of spindle fibers (fig. 10 *C*) while others appear to be formed of individual sets of spindle fibers (fig. 10 *D*) lying side by side. The daughter nuclei resulting from abnormal spindle figures are of various shapes and sizes (fig. 10 *G*). Some are considerably elongated and often amoeboid (fig. 10 *H*) in appearance, similar to the nucleus figured by Soltwedel (1882) in the endosperm of *Leucojum aestivum* L.

Previous accounts of abnormal spindle figures in endosperm nuclei fail to explain satisfactorily the origin of such abnormalities. Two explanations suggest themselves as a result of this study of fig endosperm: (1) the abnormal mitotic figures may be the result of the dense crowding of the nuclei in the multinucleate cells formed by furrowing; (2) they may be due to the polyploid nature of the nuclei. Evidence in support of the first hypothesis is to be seen in the close proximity of the dividing nuclei. Some of these division figures lie side by side with their respective spindle fibers parallel (fig. 10 *D*). Others lie almost in the same plane but with the spindles end to end (fig. 10 *E*). In still other cases the figures are contiguous but diverge at various angles (fig. 10 *F*) from one another. The daughter chromosomes migrating to the poles of these spindles may become more or less

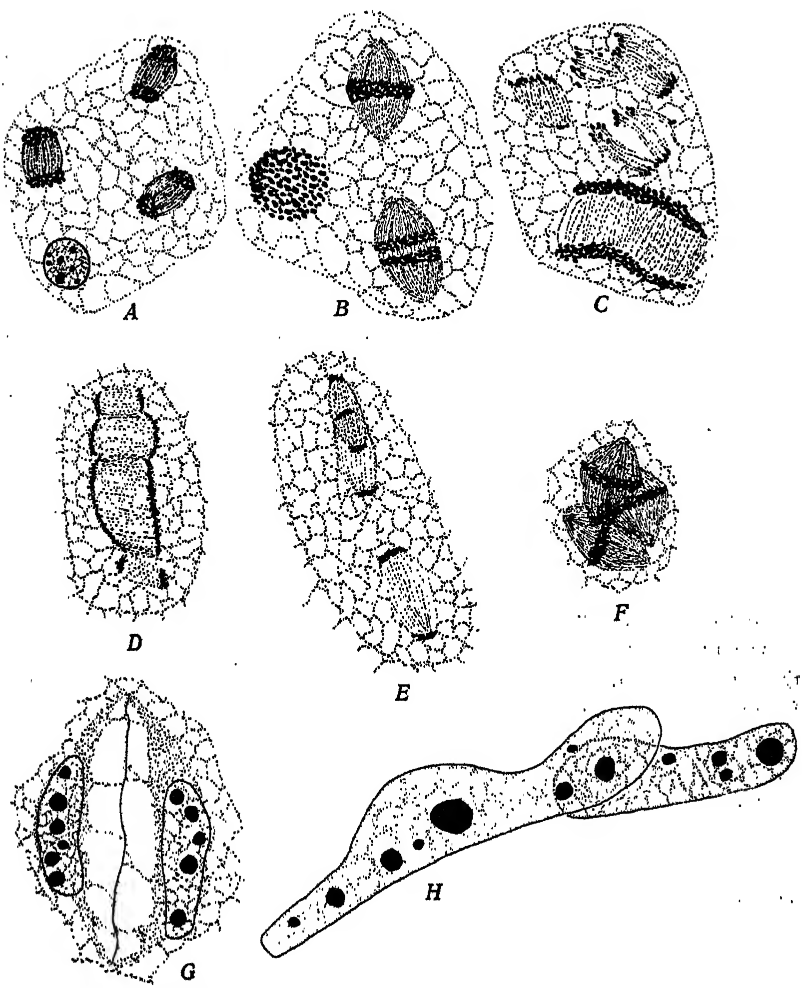

Fig. 10. *A*, Mitotic figures with 2n chromosomes and a resting nucleus characteristic of the early free-nucleate stage of parthenogenetic endosperm. (X 1,140.)
B, Mitotic figures and a metaphase plate of polyploid nuclei indicating a chromosome complement several times n. (X 1,140.)
C, A broad homogeneous spindle figure of a polyploid nucleus, and spindle figures with groups of chromosomes more or less detached. (X 1,140.)
D, An elongated spindle figure made up of individual sets of spindle fibers. (X 1,065.)
E, Spindle figures lying in almost the same plane with spindles end to end. (X 1,140.)
F, Three spindle figures more or less intermingled. (X 1,140.)
G, Elongated daughter nuclei resulting from mitosis of a polyploid nucleus. (X 1,140.)
H, An elongated polyploid nucleus amoeboid in appearance. (X 1,065.)

intermingled with one another during the early telophases, with a resultant formation of polyploid or aneuploid nuclei instead of the normal daughter nuclei. Subsequent fusions of mitotic figures bring about still greater abnormalties, the most abnormal figures being found during the later stages of endosperm development.

If the above explanation is correct, more frequent reports of abnormalties in spindle figures of endosperm nuclei would be expected, especially in species where free nuclei and their mitotic figures lie close together. In the gymnosperms, neither nuclear fusions nor irregular mitotic figures ordinarily result from crowding of free nuclei in the macrogametophyte. Reference is made above to two cases of abnormal figures in the endosperm nuclei of angiosperms, but the literature of endosperm development records very few such abnormalities.

The other hypothesis for abnormal spindle figures takes into account the possibility that the nuclei which make up a polyploid endosperm nucleus more or less retain their individuality during several successive mitoses. Fusion of resting nuclei either in the free-nucleate or in the cellular endosperm does not necessarily mean an immediate mingling of chromatin. Nothnagel (1918) has shown that in the triple fusion of a sperm nucleus and the polar nuclei to form the primary endosperm nucleus of *Lilium martagon* L., a complete fusion or intermingling of chromatin did not immediately occur, as in some cases three groups of spiremes are plainly evident at the time the fusion nucleus first divides.

The nuclear membranes of the polyploid nuclei found in the fig endosperm are not distinct, often making it difficult to determine whether nucleoli in groups are enclosed within a common membrane or are parts of individual nuclei lying in different planes. The location and arrangement of the nucleoli in elongated and amoeboid-like nuclei indicate, however, that their individuality has been maintained during mitosis. As already stated, some of the spindle figures are very definitely composed of distinct sets of spindle fibers, a fact which indicates that the mitotic figures of individual nuclei combine to make up a composite figure.

The study of these abnormal figures in fig endosperm leads me to favor the hypothesis that explains irregularity of division as due to the polyploid nature of the nuclei. It is interesting to note that Johnson (1914, fig. 106) shows a similar irregularity of division for the polyploid primary endosperm nuclei of *Peperomia hispidula* A. Dietr. that has 14n chromosomes. Further study of other material

will be necessary to establish as a certainty the assumption that nuclei more or less maintain their identity in the formation of a polyploid endosperm nucleus.

Mitoses of polyploid nuclei are, in the later stages of endosperm development, generally accompanied by the formation of very definite cell plates (fig. 10 *G*) on the spindle fibers. Although there is no question of the formation of cell plates, it is not at all certain that their presence results in cytokinesis. These cell plates may be a transitory type similar to those found in the pollen mother cells of *Larix* (Devisé, 1922).

There is a tendency for the cells in the later stages of endosperm development to become uninucleate. At this stage most of the parthenogenetic endosperm cells, but especially those at the periphery, contain numerous granules similar to but smaller (fig. 9 *B*) than those found in the cells of normal endosperm. The endosperm of short-styled gall flowers is transitory and is entirely consumed by the blastophaga larva at about this stage of its development.

Normal endosperm and parthenogenetic endosperm differ in the following particulars: In normal endosperm, nuclear fusions are not common and mitoses are generally regular; the chromosome complement is usually 3n; cell formation is brought about by cleavage; the peripheral cells are cambium-like in appearance; most cells are uninucleate and their walls are of cellulose. The parthenogenetic endosperm of long-styled flowers has diploid nuclei; cell formation, however, appears to be similar to that of normal endosperm. In the parthenogenetic endosperm of short-styled flowers, cell formation occurs by a process of simultaneous furrowing; the cells are commonly multinucleate, their outlines usually being indistinct and poorly defined; both peripheral and central cells are irregular in size and shape; nuclear fusions commonly occur, the polyploid nuclei being of various shapes and sizes; mitoses of adjoining nuclei frequently form mitotic figures very abnormal in appearance; the chromosome complement is irregular, varying from n to several times n.

EMBRYOGENY

The development of the fig embryo begins with an elongation of the zygote and a vacuolation at the micropylar end. The zygote nucleus contains a single nucleolus, although daughter nuclei are often multinucleolate.

The first transverse division of the zygote results in the formation of an embryo with a basal and an apical cell. There is then a transverse division of both cells to form a linear four-celled embryo. Usually the division of the basal cell is fully completed before there is any indication of division in the apical cell.

The basal cell or cells develop into a short suspensor, which is transitory and not more than three cells in length. Material showing subsequent stages in the development of the embryo was insufficient to work out the embryogeny in detail. However, it appears to follow the same general type of development as that described by Souges (1921) for *Urtica pilulifera* L. The young embryo of the fig is at first spherical and remains so until successive cell divisions have formed a massive embryo of several hundred cells. It then becomes flattened at the apex, after which the two cotyledons differentiate in the usual way with the epicotyl between. The mature embryo is curved so that the hypocotyl and the cotyledons lie side by side (fig. 6).

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RELATION OF SPECIFIC GRAVITY TO THE QUALITY OF DRIED PRUNES

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Aside from the general provisions of the Federal Food and Drugs Act of 1906, which deal with sanitation, adulteration, and the misbranding of foodstuffs, there are no legal standards of quality for dried prunes. The standards for quality, apart from size, used in the industry also are confusing and involved, and the specifications used are expressed in very vague terms. During the evolution of the industry, which is centered in several widely separated districts of the state, geographical names have come to be associated with, and even to represent, grades of quality. Thus there are found in trade usage such terms as Santa Clara, Northern California, San Joaquin, California, Northern Outside, Colusa, Napa, Sonoma, and Suisun. Of these, the first carries the most prestige and is applied only to fruit of the best quality, while several of the other terms are practically synonymous. The usefulness of these names as quality designations has been impaired somewhat by the recent decision of the Federal Food and Drugs authorities that geographical terms may be used in domestic trade to refer only to products of the localities named. Such general terms as Extra Fancy, Fancy, Extra Choice, Choice, and Standard, which are commonly used to designate grades of quality in other California fruit products, are not generally applied to dried prunes. The specifications for various prune grades use such indefinite terms as "a great predominance of clear amber meat,"

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"containing any material percentage of scabby, slabby, fermented or split specimens," or "fruit of a coarser, more fibrous and porous texture."[(1)][3] The grading is done by the visual inspection of individuals, who base their segregations upon the general external appearance of the fruit and the condition of the flesh as shown by cutting a few specimens. Finally, the system is complicated by being of the flexible type, varying from year to year. This is for the purpose of maintaining approximately the same proportion of the crop in each grade each year regardless of the seasonal variations in crop size and condition.

In contrast to the more general aspects of quality, size is usually expressed very specifically as the 'count' or number of fruits to the pound. Usually fruit is sized or size-graded in count ranges of 10, as 20–30, 30–40, and so on. Even in this matter, trade practice is not entirely uniform, since some packers separate the fruit into only three size groups—large, medium, and small—in each of which the range of size is 20 'points' or more.

From what has been said so far, it should be clear that the present basis of quality classification contains a large element of personal and individual judgment. In connection with some investigations of the effects of different harvesting and drying practices upon the quality of dried prunes, it became necessary to have more exact means of determining and comparing the quality of different lots. The tests to be described here were developed to meet this need and also, if they appeared satisfactory for the purpose, to form the basis for improved commercial grading methods. With respect to this second object of the work, it may be said that it is not our purpose to alter the existing commercial grades but rather to provide grading methods more reliable than those now in use.

Among the characteristics of quality in dried prunes, the color and texture of the flesh are of great importance. In fruit of best quality, the color of the flesh is amber, or lighter, and the flesh is solid in texture (fig. 1). Since good flesh color is commonly associated with solid texture in dried prunes, and vice versa, and since texture in different prunes varies all the way from solid to the extreme of porosity characteristic of 'bloaters' in which are one or more large air pockets, measuring the specific gravity of the fruit directly or indirectly was believed to be a promising method of measuring both color and texture of the flesh.

[3] Superscript numbers in parentheses refer to selected references at the end of this publication.

MATERIAL STUDIED

Since prunes of the French (Agen) variety constitute about 90 per cent of the crop in California, only this variety was studied intensively. It is believed that tests found to be of value for this variety will probably be of equal service with other varieties even though the characteristics and grade requirements may not be identical. The material used in this study was obtained from packing houses in the four principal prune-growing districts of the state.

Fig. 1. Flesh texture of prunes: *a*, solid; *b*, bloaters.

The districts were designated by letters as follows: A, the Sacramento Valley; B, the Napa-Sonoma district; C, the Santa Clara Valley; and D, the San Joaquin Valley. Districts A and D are those commonly known in the trade as the "outside districts," while B and C are known as the "inside districts" or "three districts."

From what has been stated in the introduction regarding the use of geographical names as designations of quality, it will be seen that getting prunes from each district representing each of the quality grades recognized in the industry was impossible, and it was not feasible to proceed as had been done in the raisin standardization study to which reference has been made.(2) In the raisin study, samples were obtained from each of the many receiving stations involved, and from nearly all plants samples were secured that represented each of the regular grades, namely Extra Standard, Standard, Sub-Standard, and Inferior. The experimental tests were

then applied to all samples and the results were studied, using as a group all samples placed in a single grade by visual inspection, regardless of the district from which they were secured. In the prune quality study, on the contrary, when the samples for examination were secured, the great bulk of the fruit at each packing house had been placed in the single grade characteristic of that district. Most of the samples from each district therefore represented only a single grade. Fruit in this grade, which was the first or best grade in each district, did not, however, necessarily correspond in quality to fruit in the first grade of any other district. For these reasons the samples when tested were grouped for study according to district rather than quality grade.

Two kinds of samples were studied in 1928 and 1929, namely bin samples and growers' samples. Bin samples were composite samples taken from the storage bins at packing houses. Wherever possible such samples were obtained from a trench dug from top to bottom in a bin full of fruit, after it was opened from the side. Thus the fruit in the bin samples had been graded for size and represented in each case many or all of the individual growers' lots of which the bin load was composed.

Each grower's sample, on the other hand, was from a single delivery of a grower and was 'orchard run,' i.e., not size-graded.

In 1930 most of the samples were taken from individual growers' lots before size grading. Only a few composite, size-graded samples were taken from bins, and all samples from each district were grouped together for statistical study.

EXPERIMENTAL METHODS

Methods of judging or measuring size, skin condition and moisture content will be presented in another paper. The present discussion is limited to the studies of specific gravity and weight per volume, which were found to measure directly the texture, and indirectly, the color, of the flesh.

Specific Gravity.—The presence of air pockets of considerable number or size obviously affects the relation between the size and the weight of the prunes. Two physical tests are directly affected by this condition, that of determining specific gravity and of weight per volume. Specific gravity may be determined by weighing an object in air and observing its decrease in weight when suspended in water or some other liquid of which the specific gravity is known.

Because of the great surface tension of water, which tends to catch bubbles of air on the irregular surface of prunes, a mixture of xylene and carbon tetrachloride was found to be a more suitable liquid for use in determining specific gravity. Such a liquid has several advantages. The liquid does not dissolve fruit sugars that would change its specific gravity. The presence of carbon tetrachloride reduces the inflammability of the xylene. The difference in specific gravity of the two liquids permits ready adjustment of the specific gravity of the mixture. On account of its low surface tension it readily wets all parts of the fruit, thus releasing any bubbles of air caught in the wrinkles. The specific gravity of the prunes was, therefore, determined by weighing them in air and again in the xylene mixture.

Individual prunes were studied by this method while suspended by a fine wire. These preliminary tests with individual prunes showed a close relation between specific gravity and internal condition. In the later work one-pound samples were used that, when submerged, were weighed in a wire basket. The equipment required for these tests included:

Balance, capacity 1,000 grams and sensitive to 0.1 gram, provided with a hook under one pan. Pans approximately 6 inches in diameter.
Aluminum pan, 1 liter capacity.
Wire basket, approximately 5 inches in diameter, and 5 inches tall.
Set of weights, 200 grams to 0.1 gram.
Cylindrical can approximately 7 inches in diameter and 10 inches in height. (Preferably fitted with a friction-top lid.)
Commercial xylene.
Commercial carbon tetrachloride.
Specific gravity hydrometer; scale, 1.000 to 0.800 by divisions of 0.005.

The specific gravity of the xylene is adjusted to exactly 0.900 by cautious additions of small amounts of carbon tetrachloride, followed by thorough stirring and testing with the hydrometer. The specific gravity of this mixture changes by 0.001 for each Centigrade degree change in temperature.

The balance is supported above a stand at sufficient height to permit the wire basket, hung by a piece of fine wire from the hook below one of the pans, to be completely submerged in the can filled to within 2 inches of the top with xylene. The level of the xylene should come part way up the supporting wire when no prunes are

in the basket. With the basket thus submerged, and the aluminum dish upon the pan above, the balance is brought to equilibrium by a tare weight on the other pan. (A small can of shot is convenient for the purpose.)

A one-pound sample of prunes is now placed in the aluminum dish and weighed to the nearest gram. The prunes are next transferred to the basket, the aluminum dish replaced on the pan, and the weight again taken to the nearest gram.

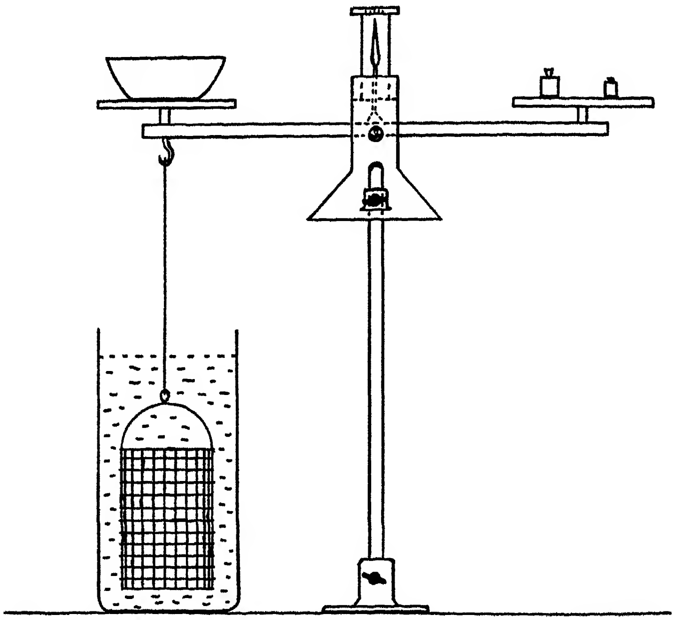

Fig. 2. Diagram of apparatus for specific gravity test.

From the weight of the prunes in air and the loss of weight in xylene (the difference between the two weights observed) the specific gravity may now be calculated by the following formula:

$$\text{Specific gravity of prunes} = \frac{\text{Weight in air} \times \text{specific gravity of xylene}}{\text{Loss of weight in xylene}}$$

The calculating may be omitted and the determination simplified by use of a direct-reading chart (fig. 3).

The specific gravity of the xylene mixture should be checked at least twice a day, and corrected if necessary.

After the determination, the prunes may be spread out in a thin layer in a well ventilated place away from open flames. After one to two days, the xylene will have evaporated completely and the prunes

will show no effect from the immersion. If the fruit is placed over a steam radiator or in a dehydrater the xylene will evaporate in a few minutes.

Weight per Volume.—Weight per volume or W/V value is determined by weighing a constant volume of the fruit in a standard container filled in a uniform manner. In the studies made during the first two years the tests were made by filling a 2-quart can with the fruit and mechanically shaking the can at a uniform rate as it

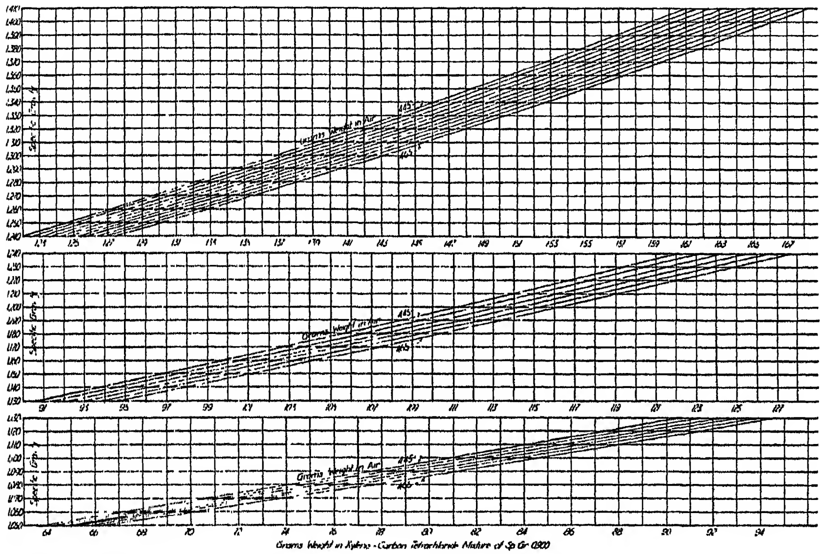

Fig. 3.—Specific gravity from weights in air and in a xylene-carbon tetrachloride mixture having a specific gravity of 0.900. To find the specific gravity (1) find on the proper base line the weight of the samples submerged in the liquid, (2) follow up the vertical line from this point to its intersection with the sloping line corresponding to the weight of the sample in air, (3) from this point of intersection proceed horizontally to the left to the scale from which the specific gravity may be estimated. Note that in the lower two portions of the chart the sloping lines representing the even numbers of grams weight in air have been omitted to avoid crowding of the lines, and the proper points of intersection must therefore be estimated.

was being filled. In 1930 a larger machine was constructed and equipped with a 5-gallon can (figs. 4 and 5) and a large number of samples were tested by both machines in order to determine the relation between the results obtained by them. This permitted the results obtained in all three years of the study to be reported on the same basis, namely, as gross weight of the 5-gallon can and contents in pounds and ounces. This is referred to as the "W/V value."

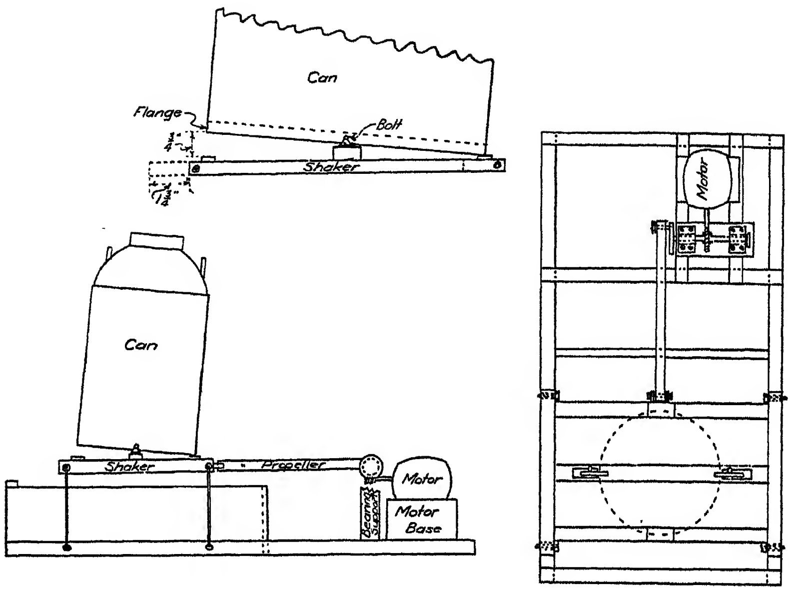

Fig. 4. Diagram of apparatus for weight per volume test.

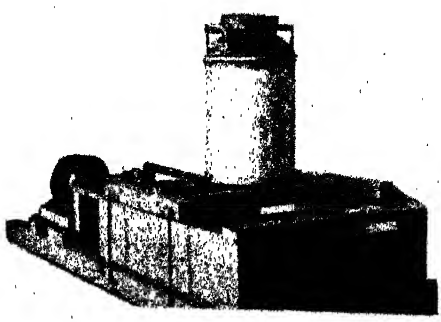

Fig. 5. Apparatus for determining weight per volume.

The essential parts of the 1930 apparatus are a standard round-shouldered 5-gallon milk can and shaker platform.

The flare of the can is cut off smooth at the top of the vertical neck. A $\frac{3}{8}$-inch hole is drilled at each of two opposite points in the vertical flange at the bottom of the can. The empty can, with the flared top cut off and without lid, weighs 11 pounds 4 ounces. Filled with water at 54° F, it weighs 54 pounds 8 ounces and thus has a capacity of 43 pounds 4 ounces of water at this temperature, equivalent to 5.07 gallons or 1,171 cubic inches.

The shaker platform has a throw of $1\frac{3}{4}$ inches and moves at the rate of 88 round-trip cycles per minute. It has a central cross member on which are installed two round sliding door-bolts $\frac{5}{16}$ inch in diameter so located as to slip into the holes in the bottom flange of the can and hold the can in position, acting as bearings for rocking the can during filling and subsequent shaking. The central cross member bearing the bolts is raised so that when the can is bolted in position and held level, the bottom of the can is $\frac{3}{8}$ inch above the level of the metal strike plates, allowing the front and back edges of the bottom of the can to move up and down a total vertical distance of $\frac{3}{4}$ inch during rocking.

The unit is driven by a 110-volt motor of $\frac{1}{4}$ horsepower running at 1760 r.p.m. A worm on the motor shaft drives a gear on the shaft that drives the shaker, giving a speed reduction of 20 to 1. The drive rod to the shaker is run by a disk at the end of the shaft driven by the motor with a pin $\frac{7}{8}$ inch off center.

The shaker platform is supported above the base by four shallow, wide U-bolts $\frac{5}{16}$ inch in diameter, two on each side. Brass bushings in the shaker platform and base provide bearing surface. This supports the shaker platform with the top approximately 12 inches above the floor. Under this platform, the walls of the base are built up, and boxes may be placed under the platform to catch the overflow of prunes.

The feeding of fruit to the can is so regulated as to fill the can, with shaker in operation, in 60 seconds and the shaking is then continued for an additional 60 seconds, meanwhile keeping the can full. If desired, an automatic time switch may be used for stopping the motor at the end of two minutes. After the motor is stopped, the can is heaped with prunes, leveled off by dragging a stick slowly and firmly across the neck of the can, and the can and contents are weighed upon scales reading in pounds and ounces.

PRESENTATION OF DATA

The data from different examinations will be discussed separately, but are in most cases tabulated together.

Effect of Size on Specific Gravity and Weight per Volume.—The results of the tests upon all bin samples of the 1928 crop were studied to determine whether there was association between size and specific gravity. Since these samples had previously been mechanically graded for size, all fruits in each sample were reasonably uniform in this respect. The samples were arranged in size-groups without regard to origin or quality, and the average size, specific gravity, and weight per volume for each group was calculated. The results are shown graphically in figure 6. Judged by these results, variations in size

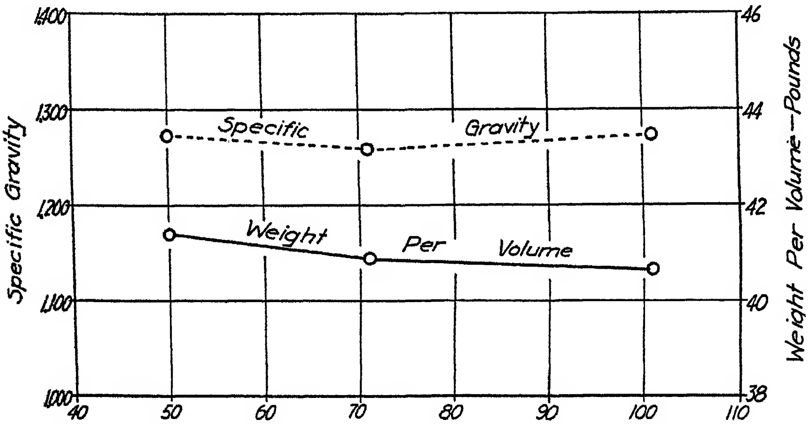

Fig. 6. Relation between size of prunes and their specific gravity and weight per volume.

alone appeared to have no consistent or important effect upon specific gravity or weight per volume. However, since the distribution of variations in quality may not have been the same in each group, the results of this study are not conclusive. A more exact estimation of the direct or indirect influence of size upon specific gravity was gained by a study of individual prunes.

Effect of Pit on Specific Gravity of Prunes.—The proportion of pit in French prunes was found to decrease as the weight or size of the fruits increased. Therefore, the influence of the proportion of pit on the specific gravity of the fruit decreased as the size increased. The

nature and extent of this influence was studied by examination of 25 prunes chosen to represent a wide range in size and in quality. The specific gravity of each whole prune was determined. The pit was removed, and the texture of the flesh was noted. The pits were carefully scraped and the proportion of pit was found. The specific gravity of each pit was also determined. From these data the specific gravity of the flesh alone was also calculated. The results are given in table 1.

TABLE 1

SPECIFIC GRAVITY AND QUALITY IN INDIVIDUAL PRUNES

Prune No.	Flesh texture	Flesh color	Count per pound	Pit, per cent	Specific gravity		
					Whole prune	Pit	Flesh
1	Solid	Medium amber	38	9.3	1.365	1.052	1.408
2	Solid	Medium amber	40	7.8	1.388	1.091	1.420
3	Medium air pockets; slightly spongy	Dark amber	38	7.7	1.355	1.065	1.385
4	Air pockets	Medium amber	47	10.4	1.360	1.085	1.401
5	Large air pockets	Dark brown	52	12 4	1.359	1.127	1.399
6	Solid	Amber	56	12.8	1.348	1.127	1.384
7	Solid	Amber	94	13.8	1.357	1.138	1.395
8	Solid	Brown	71	13.6	1.327	1.051	1.379
9	Solid	Amber	56	12.5	1.318	1.080	1.364
10	Solid	Amber	58	14.9	1.345	1.102	1.400
11	Solid	Amber	51	19.9	1.335	1.089	1.370
12	Solid	Brown	68	12.9	1.384	1.140	1.424
13	Solid	Brown	71	17.4	1.331	1.052	1.405
14	Solid	Dark amber	99	16.6	1.309	1.082	1.364
15	Solid	Dark amber	57	12.4	1.356	1.081	1.439
16	Solid	Dark brown	90	11.3	1.352	0.864	1.459
17	Solid	Amber	71	15.8	1.275	1.152	1.300
18	Solid	Dark amber	80	12.5	1.350	1.100	1.395
19	Bloater	Dark brown	36	8.6	0.962	1.070	0.953
20	Bloater	Dark brown	41	8.9	0.996	1.054	0.991
21	Large air pockets	Dark brown	27	7.8	1.295	1.055	1.320
22	Bloater	Amber	69	13.3	1.223	1.109	1.242
23	Bloater; a "mummy"	Brown	187	28.8	1.131	0.932	1.235
24	Bloater	Dark brown	116	18.6	1.084	1.121	1.075
25	Injured by insects	Dark brown	188	32.2	1.206	1.052	1.285

It will be noted that (1) the specific gravity of the pit is remarkably constant, regardless of the size or condition of the prune; that (2) the specific gravity of the whole prune and of the flesh tends to be high in the case of the solid prunes, and to be distinctly lower in the case of the bloaters and prunes with large air pockets; and that, (3) generally, dark-colored flesh is not found in solid prunes. While several exceptions to these generalizations appear, some of them have possible explanations in the light of the other characteristics shown.

The average specific gravity of the pits was 1.072 while that of the whole prunes was 1.285. The range of specific gravity of the whole prunes was from 0.962 to 1.388, a difference of 0.426. The range of specific gravity of all the pits was from 0.864 to 1.152, a difference of 0.288. Excluding prunes No. 16 and No. 23, in which the values were exceptionally low, the specific gravity of the pits ranged only from 1.051 to 1.152, a difference of 0.101, or about one-fourth the fluctuation found in the whole prunes. The range of specific gravity of the flesh of all the prunes was from 0.953 to 1.459, a difference of 0.506, or about five times as great as the usual fluctuation of the values for the pits.

The average effect of the pit was to reduce the specific gravity of the whole prunes by 0.045. The average deviation from this reduction was 0.020, so that the change was usually within the limits of 0.025 and 0.065. The effect of the pit on the specific gravity of the whole fruit is, of course, determined by the proportion of pit, the specific gravity of the flesh, and that of the pit. In large prunes, since the proportion of pit is smaller, the effect on the specific gravity is less than in small prunes. Thus in large prunes, counting 40 or fewer to the pound, the average change was 0.028; in prunes counting more than 40 but under 81 to the pound, the average change was 0.042; while in prunes counting 81 or over, the average change was 0.065. Also since the specific gravity of the pit is usually lower than that of the flesh, the numerical effect of the pit upon the specific gravity is smaller when the specific gravity of the flesh is low. As a rule, the irregularity of the specific gravity of the whole fruits resulting from the influence of the pits was within 0.020, and since this is only 5 per cent of the range for the whole fruit, it was believed that the error introduced was not significant and could be disregarded.

Sugar Content.—Chemical examination of the samples was limited to the determination of sugar content in the bin samples of the 1928 crop. The determinations were made not on the samples as a whole but upon solid-fleshed fruits only, those with air pockets being discarded in preparation for analysis because of their probable partial decomposition. The results are summarized in table 2.

These variations in sugar content are similar in character to those obtained by Hiltner and Hatherell[4] and by Cruess and Gale[3] on other material.

An association between sugar content and specific gravity has been observed by Wiegand and Bullis[7] in a report published since this work was begun. That there is a general association between

sugar content and both specific gravity and weight per volume is shown in table 2. The specific gravity and W/V value tend to be high when the sugar content is high. However, the decomposition of but a very slight amount of sugar might produce gas pockets, bad flesh texture, and color, and markedly affect the specific gravity and the W/V value without appreciably affecting the percentage of sugar present. Therefore, the sugar content does not offer a satisfactory criterion of quality in prunes.

TABLE 2

SPECIFIC GRAVITY, WEIGHT PER VOLUME, AND PERCENTAGE OF SUGAR OF BIN SAMPLES OF THE 1928 CROP

District	Number of samples		Specific gravity	Weight per volume		Total sugar
				pounds	*ounces*	*per cent*
A	17	Average	1.231	40	5	41.3*
		Maximum	1.301	42	3	49.2
		Minimum	1.174	38	10	37.9
B	11	Average	1.329	41	15	48.2†
		Maximum	1.429	42	13	52.4
		Minimum	1.270	40	12	44.3
C	12	Average	1.312	42	0	53.6
		Maximum	1.352	41	11	56.1
		Minimum	1.276	39	15	49.7
D	11	Average	1.215	39	6	40.0‡
		Maximum	1.275	41	0	42.7
		Minimum	1.138	37	6	38.1
All districts	51	Average	1.268	40	14	45.5
		Maximum	1.429	43	8	56.1
		Minimum	1.138	37	6	37.9

* 16 samples. † 8 samples. ‡ 10 samples.

Note: Total sugar is reported as invert, and is calculated to a basis of 20 per cent moisture in the flesh of the fruit. Determinations made by B. L. Hatherell and C. D. Fisher, Dried Fruit Association of California.

Observations on Specific Gravity.—The maximum and minimum values for specific gravity found for all three seasons were 1.429 and 1.122, a range of 0.307. For individual fruits, the specific gravity extended from 0.962 to 1.395, a range of 0.433.

The results on individual prunes are summarized in table 1. Those on larger samples are shown in tables 3, 4, and 5. Tables 3 and 4, unlike tables 5 and 6, include data on count per pound, and the percentage of fruits found by visual tests to be off color, of normal external appearance, bloaters (porous flesh), or of bad flesh color.

TABLE 3

RELATION OF SPECIFIC GRAVITY AND WEIGHT PER VOLUME TO QUALITY OF PRUNES OF THE 1928 CROP

District	Kind of sample	Number of samples		Count per pound	External appearance: Fruits off color	External appearance: Fruits normal*	Weight per volume		Specific gravity	Bloaters†	Bad flesh color
					per cent	per cent	pounds	ounces		per cent	per cent
A	Bin	17	Average	75	19.2	54.3	40	5	1.231	20.1	21.0
			Maximum	124	36.8	79.0	42	3	1.301	52.2	40.8
			Minimum	48	10.2	33.3	38	10	1.174	6.5	8.3
B	Bin	11	Average	68	14.4	67.2	41	15	1.329	4.2	7.9
			Maximum	122	35.1	82.9	42	13	1.429	20.0	26.2
			Minimum	48	2.0	46.9	40	12	1.270	0.0	0.0
	Growers'	20	Average	60	2.7	39.7	41	15	1.324	35.5	10.5
			Maximum	87	35.1	74.9	43	15	1.369	84.7	26.4
			Minimum	38	0.0	0.0	40	3	1.174	0.0	0.0
	All samples	31	Average	63	6.9	49.5	41	15	1.324	24.3	9.8
			Maximum	122	35.1	82.9	43	15	1.429	84.7	26.4
			Minimum	38	0.0	0.0	40	3	1.174	0.0	0.0
C	Bin	12	Average	82	14.3	63.1	42	0	1.312	2.1	10.1
			Maximum	115	38.0	87.9	41	11	1.352	6.2	20.5
			Minimum	48	2.1	36.9	39	15	1.276	0.0	0.0
	Growers'	86	Average	67	8.3	33.4	42	8	1.301	32.3	25.6
			Maximum	132	73.5	86.9	44	6	1.385	79.2	66.2
			Minimum	32	0.0	0.0	39	15	1.132	0.0	0.0
	All samples	98	Average	68	9.0	37.1	42	4	1.302	28.5	23.7
			Maximum	132	73.5	87.9	44	6	1.385	79.2	66.2
			Minimum	32	0.0	0.0	39	15	1.132	0.0	0.0
D	Bin	11	Average	82	17.2	65.7	39	6	1.215	30.5	20.4
			Maximum	121	37.3	84.0	41	0	1.275	56.7	35.6
			Minimum	45	4.3	49.5	37	6	1.138	17.1	10.0
	Growers'	8	Average	76	17.2	54.4	40	2	1.214	53.6	25.3
			Maximum	115	75.1	84.5	42	2	1.271	91.5	47.9
			Minimum	59	0.0	26.4	37	14	1.136	12.2	2.7
	All samples	19	Average	80	17.2	61.0	39	12	1.215	40.2	22.5
			Maximum	121	75.1	84.5	42	2	1.275	91.5	47.9
			Minimum	45	0.0	26.4	37	6	1.136	12.2	2.7
All districts	Bin	51	Average	77	16.6	61.7	40	14	1.268	14.7	14.5
			Maximum	124	38.0	87.9	43	8	1.429	56.7	40.8
			Minimum	45	2.0	33.3	37	6	1.138	0.0	0.0
	Growers'	114	Average	66	8.0	37.0	41	11	1.324	35.3	23.6
			Maximum	132	75.1	86.9	44	6	1.385	91.5	66.2
			Minimum	32	0.0	0.0	37	14	1.132	0.0	0.0
	All samples	165	Average	70	10.6	43.9	41	3	1.290	28.3	20.7
			Maximum	132	75.1	87.9	44	6	1.429	91.5	66.2
			Minimum	32	0.0	0.0	37	6	1.132	0.0	0.0

* From this designation were excluded fruits defective in respects other than color.

† The term "bloaters" as used here includes not only the puffed, nearly spherical fruits called bloaters in the industry, but also fruits with a few large or many medium-sized air pockets and those with spongy or woody tissue of low specific gravity.

TABLE 4

RELATION OF SPECIFIC GRAVITY AND WEIGHT PER VOLUME TO QUALITY OF PRUNES OF THE 1929 CROP

District	Kind of sample	Number of samples		Count per pound	External appearance: Fruits off color	External appearance: Fruits normal*	Weight per volume		Specific gravity	Bloaters†	Bad flesh color
					per cent	per cent	pounds	ounces		per cent	per cent
A	Bin	12	Average	80	13.5	45.4	41	6	1.292	15.5	15.4
			Maximum	113	29.2	60.4	42	8	1.377	45.3	31.3
			Minimum	47	0.0	31.9	40	8	1.242	2.9	0.0
	Growers'	45	Average	55	9.1	57.5	41	4	1.259	24.5	5.3
			Maximum	82	37.8	76.5	43	3	1.345	27.5	30.0
			Minimum	36	0.0	32.2	38	8	1.163	0.0	0.0
	All samples	57	Average	60	10.1	55.0	41	4	1.266	22.6	7.5
			Maximum	113	37.8	76.5	43	4	1.377	57.5	31.3
			Minimum	36	0.0	31.9	38	8	1.163	0.0	0.0
B	Bin	4	Average	77	10.8	52.1	42	10	1.295	5.9	2.7
			Maximum	99	18.6	58.5	43	3	1.332	11.0	6.9
			Minimum	53	5.9	41.2	42	3	1.252	1.9	0.0
	Growers'	41	Average	60	15.2	50.4	41	13	1.293	9.5	1.7
			Maximum	107	38.6	88.9	43	12	1.338	19.1	13.2
			Minimum	35	0.0	2.6	41	1	1.241	1.8	0.0
	All samples	45	Average	61	14.8	50.6	42	1	1.293	9.2	1.8
			Maximum	107	38.6	88.9	43	12	1.338	19.1	13.2
			Minimum	35	0.0	2.6	41	1	1.241	1.8	0.0
C	Bin	14	Average	81	10.2	50.4	43	0	1.304	4.3	6.1
			Maximum	134	21.8	57.5	45	12	1.345	10.2	18.3
			Minimum	49	0.0	41.4	41	12	1.228	0.0	0.0
	Growers'	25	Average	64	4.0	60.2	41	13‡	1.312	9.0	5.4
			Maximum	89	16.7	79.6	....	...	1.396	29.1	26.6
			Minimum	40	0.0	36.0	....	....	1.218	0.0	0.0
	All Samples	39	Average	70	7.2	56.7	42	13	1.308	7.3	5.6
			Maximum	134	21.8	79.6	45	12	1.396	29.1	26.6
			Minimum	40	0.0	36.0	41	12	1.218	0.0	0.0
D	Bin	15	Average	80	10.3	54.0	41	13	1.270	24.6	9.0
			Maximum	126	25.8	65.9	47	5	1.331	43.1	31.4
			Minimum	46	1.6	40.5	39	9	1.220	14.4	1.3
	Growers'	53	Average	72	9.5	49.4	....	....	1.248	30.1	10.1
			Maximum	101	26.8	75.2	...	....	1.302	62.9	30.5
			Minimum	35	0.0	20.6	....	....	1.122	13.0	0.0
	All samples	68	Average	73	9.7	50.7	42	3	1.252	28.9	9.8
			Maximum	126	26.8	75.2	47	5	1.331	62.9	31.4
			Minimum	35	0.0	20.6	39	9	1.122	13.0	0.0
All districts	Bin	45	Average	80	11.2	50.4	42	3	1.290	14.2	9.3
			Maximum	134	29.2	65.9	47	5	1.377	45.3	31.4
			Minimum	46	0.0	31.9	39	9	1.220	0.0	0.0
	Growers'	164	Average	65	9.4	53.5	41	8	1.272	20.2	6.0
			Maximum	107	38.6	88.9	43	12	1.396	62.9	30.5
			Minimum	35	0.0	2.6	38	8	1.122	0.0	0.0
	All samples	209	Average	68	9.8	52.8	41	13	1.275	18.9	6.7
			Maximum	134	38.6	88.9	47	5	1.396	62.9	31.4
			Minimum	35	0.0	2.6	38	8	1.122	0.0	0.0

* See first footnote, table 3. † See second footnote, table 3. ‡ Only one sample.

TABLE 5

RELATION OF SPECIFIC GRAVITY AND WEIGHT PER VOLUME TO QUALITY OF PRUNES OF THE 1930 CROP

District	Number of samples		Defects, external: Major*	Defects, external: Weighted†	Defects, external: Total‡	Weight per volume		Specific gravity
			per cent	per cent	per cent	pounds	ounces	
A	23	Average	17.1	19.2	30.8	40	13	1.273
		Maximum	42.4	43.7	60.0	42	15	1.338
		Minimum	9.0	10.1	12.0	38	12	1.178
B	30	Average	11 8	14.5	27.6	41	14	1.290
		Maximum	29.0	29.5	55 0	45	9	1.340
		Minimum	3.0	5 4	9.0	38	15	1.228
C	21	Average	10.3	12.9	25.4	40	15	1.300
		Maximum	19.4	21.8	40.0	42	14	1.347
		Minimum	4.0	6.2	17.0	39	9	1.252
All	74	Average	13.0	15.7	28.0	41	7	1.288
		Maximum	42.4	43.7	60.0	45	9	1.347
		Minimum	3.0	5.4	9.0	38	12	1 228

* Percentage of fruits with important defects only.
† Minor defects included but given reduced weight in the percentages.
‡ Both major and minor defects included and given the same weight in the percentage.

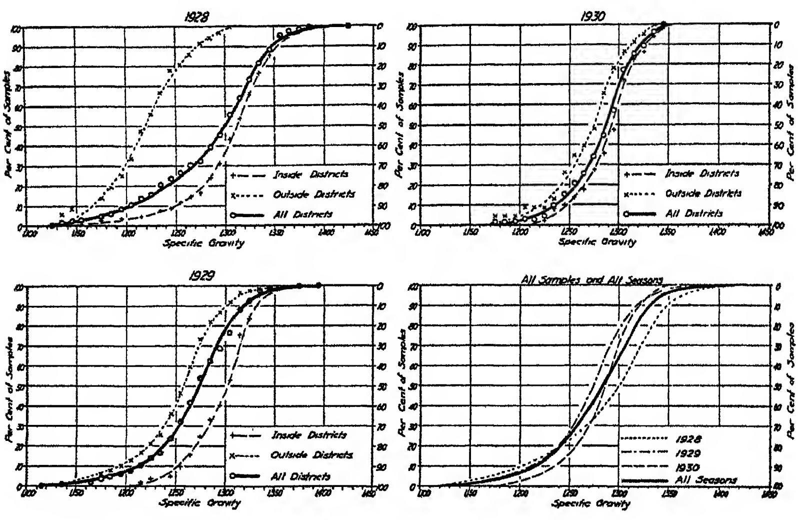

Fig. 7. The distribution curves of specific gravity among samples of the 1928, 1929, and 1930 crops.

The proportion of samples found to be of various specific gravities in the range observed is shown graphically in figure 7. It will be noted that the average specific gravity of the samples from the inside districts (B and C) was higher for all three seasons than of those from the outside districts (A and D). There was, however, considerable overlapping of values. Thus, as shown in figure 7, 50 per cent of all samples in 1930 had specific gravities below 1.29; only 40 per cent of the samples from the inside districts had specific gravities below this value, while 60 per cent of the samples from the outside district were below 1.29 in specific gravity.

The average and range of specific gravity of samples from all the principal districts are shown for the three years in tables 3, 4, and 5. It will be seen that seasonal variations occurred, but these were not large, and the average for each district remained in a range that is characteristic of the district. Also, the values in districts A and D were similar to each other but lower than those of districts B and C, which were also similar to each other.

Effect of Moisture Content on Specific Gravity.—In order to determine the effect of change in the moisture content upon the specific gravity of prunes, two samples each of about 20 per cent moisture content were tested, then dehydrated to moisture contents of about 10 per cent. Increases of 0.016 and 0.045 in the specific gravity appeared when the samples were again tested, using the same fruits. Using the observed specific gravities and moisture contents, the calculated changes in specific gravity were approximately 0.037 and 0.033, respectively. In the first case the specific gravity change observed was 0.021 less than that calculated, indicating probably that the air pockets of the fruit became relatively larger during drying. In the second case the specific gravity change observed was 0.012 more than that calculated, indicating that the air pockets became relatively smaller during drying. The fact that such small changes occurred as a result of such large changes in moisture content was taken to indicate that variations in moisture content did not impair the usefulness of the method. The changes in moisture content in the experiment were approximately twice the normal range of moisture content of prunes delivered to packing houses. Also, since the specific gravity decreases as the moisture content increases, any changes in the specific gravity from this cause is in the right direction; the value of the fruit is less at higher moisture content.

Effect of Temperature on Specific Gravity.—A similar study was made of the effect of temperature upon the specific gravity. Ten

samples were tested at 70° F, and again after the temperature had been adjusted to 32° F. The change in temperature had but slight effect upon the specific gravity, the average increase in the reading being about 0.01 for this difference in temperature. Since this variation in temperature was as great as any to be expected in commercial practice, it was concluded that the effect of temperature on specific gravity could be ignored.

Observations on Weight per Volume.—The weight per volume test was made upon all samples of sufficient size. The results are summarized with other data in tables 3, 4, and 5. The W/V value, like the specific gravity, was found to increase with increase in the proportion of fruits having solid texture and good color in the flesh. The extreme range of W/V values found in all the samples studied for the three seasons was from 37 pounds 6 ounces to 47 pounds 2 ounces, a difference of 9 pounds 12 ounces.

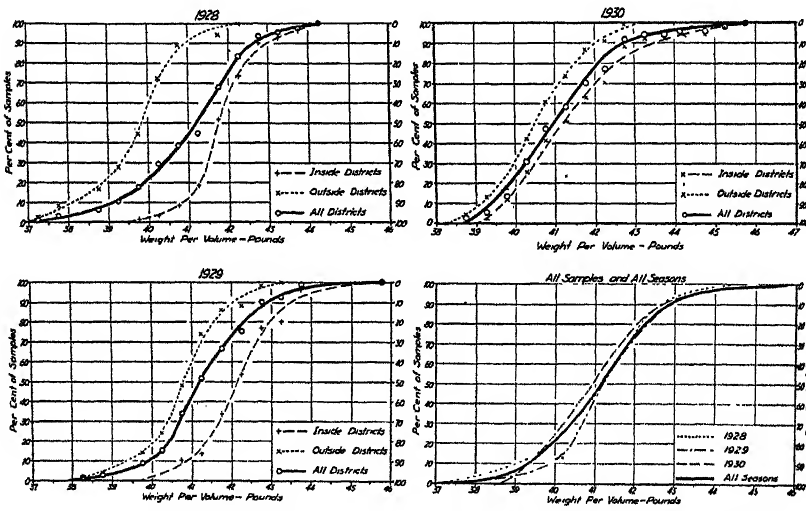

Fig. 8. The distribution curves of weight per volume among samples of the 1928, 1929, and 1930 crops.

The average W/V values were higher in the inside districts B and C than in the outside districts A and D. The proportion of samples occurring at different weights per volume is shown in figure 8. It will be noted that the overlapping of values among samples from the different districts is very much like that in figure 7. Thus, in 1930, 50 per cent of all samples studied showed a weight per volume of less than 41 pounds. Of the samples from the inside districts, only

45 per cent tested less than 41 pounds, while 67 per cent of the samples from outside districts had a W/V value of less than 41.

The influence of moisture content upon the W/V value was found to be slight but less regular than upon the specific gravity. Of three wet samples tested before and after further drying, two gained 2 and 3 ounces, respectively, while one lost 8 ounces in weight per volume during drying to the lower moisture content.

Effect of Temperature on W/V Value.—Temperature had a much more pronounced effect upon weight per volume than upon specific gravity. Three samples tested at 70° and at 32° F increased by 1 pound 7 ounces, 1 pound 11 ounces, and 1 pound 12 ounces, respectively, in W/V value in changing from the higher to the lower temperature.

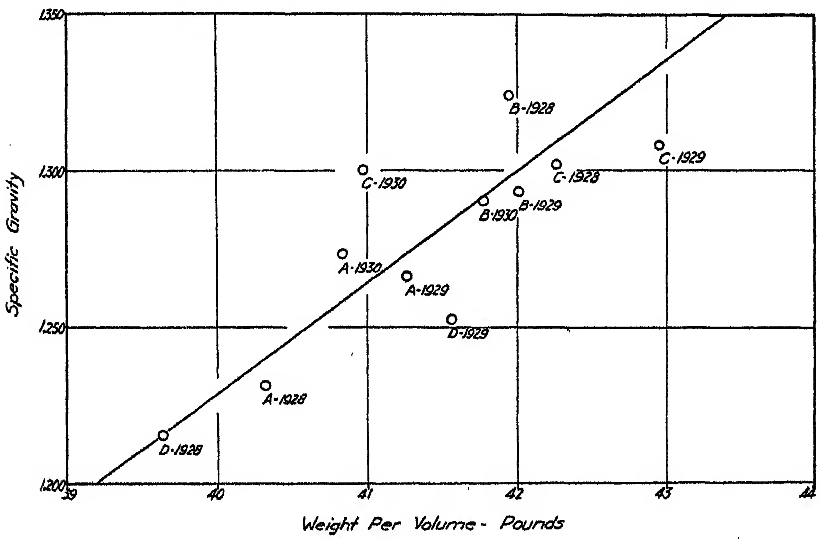

Fig. 9. Relation between average weight per volume and specific gravity of prunes for different localities and different seasons.

These differences were too great to appear to be the result of contraction of the fruit. It was noted that the fruit was less sticky at 32° than at 70° F. The higher W/V value at the lower temperature was attributed to changes of viscosity of the sirup on the surface of the fruit. This change could be assumed to affect the settling of the fruit in the container.

To determine the effect of stickiness a sample of very sticky fruit was subjected to the W/V test. It was then dipped in small portions in water at about 140° F and quickly dried first with towels and then for a few minutes in a dehydrater. Such treatment could have little

or no effect on the moisture content. After the fruit had been restored to room temperature it was tested again. It was no longer sticky. The W/V value before washing was 42 pounds 11 ounces; that after washing was 44 pounds 1 ounce. It appeared, therefore, that the removal of the stickiness without other change in the character of the fruit resulted in better settling in the test can, and thus an increased weight per volume. This was thought to confirm the hypothesis that changes in weight per volume resulting from changes in temperature were principally due to the effect of temperature upon the stickiness of the fruit.

This factor of stickiness is thought to account for the fact that the association between specific gravity and weight per volume of the samples was not closer. A study of tables 3, 4, and 5 shows that these tests did not always vary in the same direction, even among the average values, and this is shown graphically in figure 9. The degree of conformity, if individual determinations were compared, was much less. Yet there can be no doubt that both the specific gravity and the weight per volume are affected directly by the porosity of the flesh of the fruit.

DISCUSSION OF RESULTS

Two tests were developed for measuring the quality of the flesh. The color of the flesh was found to be closely associated with the texture, and the tests were based upon the latter, since it can be more easily and accurately measured. Both tests, namely, specific gravity and weight per volume, chiefly reflect the fact that increasing porosity of the flesh decreases the weight of a prune of given size or volume. The specific gravity test appeared to be least affected by interfering characteristics, chief of which was the stickiness of the fruit. Since stickiness was influenced by temperature, misleading results may be obtained by the weight per volume test unless it is made at an approximately constant temperature. Tests on sticky fruit would probably be affected more than would those of fruit relatively free of stickiness. For this reason testing fruit at different temperatures and applying a temperature correction would seem inadvisable and inaccurate. Therefore, the specific gravity test was thought to reflect more exactly the texture of the flesh if the inconvenience of temperature adjustment were to be avoided. On the other hand, the weight per volume test had the advantages of using larger samples, of not affecting the immediate usefulness of the fruit

in the sample tested, and of discriminating against sticky fruit. In view of these facts the relative usefulness of the two tests could be determined only by practical considerations not studied by us, although, as a strict indication of texture alone, the specific gravity test was considered more reliable.

Within each of the four principal prune-producing districts from which samples were studied, the annual variations of average specific gravity and W/V value observed were not great with respect to the extent of the range of values found among individual samples. This is apparent from tables 3, 4, and 5 and figures 7 and 8. It seems to indicate that allowances for seasonal variation would not be necessary, and that permanent or semi-permanent standards could be established. From this point of view the nature of the test described herein, together with the test for size, skin condition, and moisture content, to be discussed by the authors in another paper, would make possible a system of grading having certain distinct improvements over the one now in commercial use. A system of the latter type, based as it is on rather indefinite specifications and dependent on trained individual judgment, is capable of classifying a crop into but few grades. This is not a natural classification, for it is obvious that fruit from different orchards and growers must vary through a continuous range of quality from the best to the poorest. A large number of deliveries, therefore, must vary by but the most minute degree, which the judgment of different inspectors is manifestly incapable of recognizing. From this fact arises the principal inherent objection to the commercial system now in use and the principal advantage of a specific, mechanical system. Through use of the latter the crop may be graded in a much larger number of quality classifications more closely related to the slight changes of quality naturally occurring.

SUMMARY AND CONCLUSIONS

Examination of a large number of samples of California French prunes chosen to represent the principal prune-growing sections of the state over a period of three years showed that:

Flesh texture and color are correlated with, and can thus be measured by, the specific gravity.

Flesh texture and color are also measured by weight per volume. While this test is affected by stickiness of the fruit, this theoretical objection to the test may be a practical advantage since it penalizes abnormally sticky fruit.

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THE CONTROL OF THE CITROPHILUS MEALYBUG, PSEUDOCOCCUS GAHANI, BY AUSTRALIAN PARASITES[1,2]

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INTRODUCTION

The general disappearance of injurious infestations of citrophilus mealybug, *Pseudococcus gahani* Green, in California, is attributed to the work of *Coccophagus gurneyi* Compere and *Tetracnemus pretiosus* Timberlake, two internal parasites introduced into California in 1928 from Sydney, Australia, by the University of California Citrus Experiment Station. Since 1929, after the general establishment of these parasites, the mealybug has been scarcer than at any other time since it became a major pest. This scarcity of mealybugs has been continuous without appreciable annual fluctuations. No damage has been reported in the areas where the parasites have been established for a period of about two years, nor has it been necessary to liberate *Cryptolaemus montrouzieri* Mulsant to prevent the citrophilus mealybug from increasing to injurious numbers.

The saving resulting from the work of *Coccophagus* and *Tetracnemus* may be estimated from the saving in Orange County, where more than 40,000 acres of citrus were infested and where surveys show that the parasites have prevented the recurrence of infestations that were estimated to be costing the growers $500,000 to $1,000,000

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annually. This saving is for that county only and is aside from the cost of *Cryptolaemus* production. In Los Angeles County, and other counties along the coast, the saving has been less than in Orange County. The infestations were less extensive and generally less severe in the other counties because of unfavorable climatic factors which tend to retard the development of the citrophilus mealybug.

Prior to the introduction of *Coccophagus* and *Tetracnemus* the mealybug situation was not entirely satisfactory. By 1929 approximately 75,000 acres of citrus were infested by the citrophilus mealybug. Over the greater part of this acreage the infestations were kept in check by *Cryptolaemus;* but, in spite of the good work of *Cryptolaemus,* each year an increasing number of severe infestations developed. The degree of control obtained by the use of insectary-grown *Cryptolaemus* compared favorably with the results obtained by spraying and fumigation against other citrus pests. In addition the biological method had the advantage of being comparatively cheap, the *Cryptolaemus* production for the entire infested acreage costing only $125,000 annually as compared with $35 to $40 per acre for spraying or fumigating of citrus infested with red scale or black scale. A direct comparison of the cost of controlling citrophilus mealybugs on citrus by *Cryptolaemus* with the cost of mechanical control cannot be made, for, regardless of cost, neither fumigation nor spraying has satisfactorily controlled mealybugs on citrus in California.

In 1927 some of the leading orchardists resorted to water-washing as a means of preventing the damage that results from serious mealybug infestations. Some 15 or 20 growers equipped their orchards with expensive systems of water pipes for washing the infested trees, and a concern in Santa Ana was engaged in manufacturing and installing water-washing equipment in citrus orchards. The situation made it imperative that a search be undertaken, having as its objective the discovery of additional natural enemies of the citrophilus mealybug.

THE SEARCH FOR THE NATIVE HOME OF PSEUDOCOCCUS GAHANI

The possibility of obtaining effective internal parasites of the citrophilus mealybug to bring about a more satisfactory natural control had been long recognized by entomologists engaged in biological control work in California. In 1927, when the mealybug situation became alarming, the Citrus Experiment Station of the University of California decided to send a collector abroad in an effort to secure

additional natural enemies of mealybugs. This project was under the direction of Harry S. Smith, and Harold Compere was assigned to make the trip.

The citrophilus mealybug was obviously an introduced pest, but the country of origin was unknown to entomologists. It was known to occur in the British Isles, where it was first described in 1915, but the evidence indicated that it was a recent introduction and not indigenous there. G. F. Ferris (1927) recorded the discovery of the citrophilus mealybug in South Africa. It was taken there under circumstances which led him to state that there was not any likelihood that South Africa was its native home. C. P. Clausen had searched unsuccessfully for the citrophilus mealybug in China, Japan, the Philippine Islands, and Formosa. Silvestri, then employed by the University of California, covered much of the territory explored by Clausen, and in addition Indo-China, without finding this mealybug. S. I. Kuwana, chief entomologist of the Japanese Empire, who is a specialist on the Coccidae of the Orient, had never found the citrophilus mealybug. Southern Europe was excluded as a possible place of origin, because it did not seem probable that the mealybug could have existed there without having been discovered. The occurrence of this pest in widely separated countries was sufficient grounds for the belief that it had been accidentally transported by commerce. It was assumed that the citrophilus mealybug originated in a country having a subtropical climate comparable to the coastal area of southern California and one which was closely linked by steamer transportation with California, South Africa, and the British Isles. On the basis of this reasoning it seemed that Australia was a likely place in which to search for the native home of this species. The climate of Sydney does not differ greatly from that of southern California and it is one of the world's greatest shipping centers, having regular and direct steamer communication with California, South Africa, and the British Isles.

Discovery of Pseudococcus Gahani in Australia.—On September 27, 1927, Compere discovered seven overwintering citrophilus mealybugs wedged between the scales of unopened buds on a *Choisya ternata* growing in the Sydney Botanical Garden. This early discovery was rather remarkable; for it was made shortly after beginning a plant-to-plant inspection of the garden on the first day spent in searching. It was not until several weeks later that additional specimens of the citrophilus mealybug were discovered on other host plants; the species was never again collected on *Choisya*. The collector of parasites of Coccidae usually first examines plants in botanical

gardens, estates, and residential areas where various assortments of plants are grown, for in such places there is usually found a representative assemblage of local and introduced coccids, as well as their parasites.

The discovery of *Pseudococcus gahani* Green in Sydney immediately brought up the question as to whether it was native there or a recent accidental introduction. If native it would presumably be attacked by specific parasites. Parasitism was first found on October 21, 1927, when a mealybug that had been cleared and stained was being studied microscopically. The stained mandibles and head capsule of a parasitic larva were clearly revealed through the transparent integument of the mealybug. The identity of this larva was never determined. It was not one of the species introduced into California. Possibly it was the larva of *Anusoidea comperei* Timberlake.

Control by Parasites—The evidence is now fairly conclusive that in Australia the citrophilus mealybug is held in check through the influence of parasites. When it became apparent that *Pseudococcus gahani* was very scarce in Sydney, the possibility that an adverse climatic factor might be mainly responsible for its scarcity was considered. It was felt that the finding of a heavy infestation fully exposed to the weather would eliminate this possibility. The cottony cushion scale, *Icerya purchasi,* the outstanding example of a pest controlled by natural enemies, offered a clue. In California occasional severe infestations of cottony cushion scale develop on isolated plants located in places not readily reached by natural enemies. These sporadic, isolated infestations usually develop in places out of the usual range of insects, such as on dooryard or porch plants, or on plants in hotel lobbies in the business or industrial sections of large cities.

This knowledge of *Icerya* prompted a search in the center of Sydney's industrial district, seemingly the most unlikely place to repay the visit of an insect collector. On the first day's search, an acacia tree was found which was heavily infested with cottony cushion scale. This seemed to justify the belief that possibly a severe infestation of *Pseudococcus gahani* could be found in the same area. On the second day's search, January 13, 1928, an old mulberry tree in the yard of a small dwelling at 192 Bulwara Road, Pyrmont, was found to be heavily infested with citrophilus mealybug. The tree was fully exposed to wind and rain. Cottony secretions hung in festoons from the under sides of the limbs, and the ground beneath the tree was sprinkled with gravid mealybugs which had either migrated

or fallen from the tree. A photograph of this tree, taken after three of the largest limbs had been removed and packed for shipment to California, is shown in figure 1. When the tree was found, no evidence of parasitism was noticed, and the mistake was made of supposing it to be an infestation free of parasites because of its isolation and inaccessibility.

Fig. 1. Mulberry tree, Bulwara Road, Sydney, that was heavily infested with *Pseudococcus gahani* Green. (The photograph was taken after infested limbs were removed.)

An Attempt to Secure Parasites by the Use of Traps.—Prior to the discovery of the heavy infestation of *Pseudococcus gahani* in January, comparatively few specimens of mealybugs were collected. When it became apparent that only occasional parasitized *P. gahani* could be found under natural conditions, an attempt was made to attract parasites to mealybugs on plants which had been purposely infested in the laboratory and then placed in the open in proximity to plants growing in places where the parasites were known to occur. Twelve small oleanders in pots were stocked with mealybugs. When the plants were thoroughly infested they were placed in various districts under shrubs and trees where mealybugs had previously been collected. One week later, when the first inspection was made, it was found that all the mealybugs had disappeared from the trap plants.

The disappearance was attributed to heavy rains accompanied by high winds, which were thought to have dislodged the mealybugs and washed them away.

Propagating the Parasites.—Plans were made to operate a small insectary when the first specimens of *Pseudococcus gahani* were discovered on September 27, 1927. Sprouted potatoes, trays, cages, and the usual paraphernalia necessary for propagating mealybugs were obtained. Of the seven mealybugs discovered on the *Choisya ternata*, three of the smallest were left undisturbed so that they could reproduce on the plant; the two largest specimens were prepared for microscopic study, to verify the field determination; and two specimens were placed on a potato sprout and confined in a jar so that they could reproduce.

As the season advanced and the weather became warmer, scattered specimens of *Pseudococcus gahani* were collected on certain grevilleas and oleanders in the Botanical Garden. These specimens were usually found associated with the long-tailed mealybug, *P. longispinus* (Targ.). *P. gahani* was rare; on some days no specimens were found, and at other times four or five specimens were obtained as the result of a day's collecting. Searching for a period of several weeks in the citrus orchards within a 25-mile radius of Sydney resulted in the collection of a total of only 69 specimens on citrus, practically all of which were taken singly.

Except for occasional specimens that were prepared for microscopic study, all healthy-looking mealybugs were placed on potato sprouts in cages in the laboratory. If the mealybugs showed signs of parasitism they were isolated in vials. There was no way of detecting and segregating the mealybugs which contained eggs or young larvae of parasites. Because of this, some parasitized mealybugs were introduced into the breeding stock. Adult parasites eventually issued from the parasitized mealybugs placed in the cages. All parasites were captured and transferred to separate jars or cages as soon as they were seen. At the time it was thought that this procedure would maintain a balance between the numbers of parasites and their hosts and that the stock of both parasites and mealybugs could be kept indefinitely. However, the efficiency of the parasites and the rapidity with which they could breed were not fully appreciated. Some of the parasites oviposited in the mealybugs reserved for propagation before they were captured and removed to separate cages.

About the first week in January, 1928, it became apparent that too many parasites had been permitted to develop and that all the cages reserved for propagating mealybugs were infested with them. The

situation was further complicated by excessive rotting of the potatoes which were depended upon for propagation of the mealybugs. A continuous supply of mealybugs could not be maintained to carry the parasites generation after generation without a continuous supply of potato sprouts. Ten sacks of certified, supposedly disease-free potatoes had been placed in cold storage to be used when mature potatoes were no longer available and before potatoes of the new crop would sprout. When the cold-storage warehouse was visited for the purpose of getting a sack of potatoes, it was found that the potatoes were almost a total loss as a result of rots of the most virulent types. Enough sprouts were available in January to continue the work for a month or six weeks. Because of horticultural quarantine restrictions, green lemons or other fruits could not be used to propagate mealybugs destined for shipment to California. The finding of a large quantity of mealybugs on the mulberry tree, January 13, 1928, temporarily provided plenty of hosts for the parasites that had accumulated, but not enough potato sprouts were available to care for the mealybugs from this source.

Transporting the Parasites.—Because of the presence of diseased potatoes scattered through all the cages, the material was considered unfit for a long-distance shipment in tightly closed boxes. Several possible ways of shipping the parasitized mealybugs to California were considered, but the best plan seemed to be that of personally transporting the entire lot of material on the first steamer leaving for the United States. The decision in favor of this plan was influenced by the fact that an immediate departure would make it possible to transport the entire stock safely at one time. The other alternative was to make small shipments while at the same time endeavoring to preserve a breeding stock at Sydney throughout the winter months without sufficient host material. The latter plan would have necessitated the destruction of a major portion of the natural enemies to preserve a balance between parasites, mealybugs, and potato sprouts; while the former plan offered the inducement that if the parasites should prove successful in California their establishment in large colonies at an early date would be a decided gain. Approval of the plan to return to California was secured by cable, and arrangements were made to depart on the steamship Tahiti, February 23, 1928.

On February 20, the infested tree at Bulwara Road was visited with the supposition that no parasites occurred there, and with the expectation of obtaining a quantity of unparasitized mealybugs to be used as hosts for the parasites while in transit to California. On the previous visits no parasitism was observed in the mealybugs collected

from the ground or from the low-growing shrubs under the mulberry tree. However, the final inspection revealed a condition quite different from that anticipated, because what appeared when viewed from the ground to be trailing festoons of male pupae and egg masses proved to be the mummified bodies of countless thousands of parasitized immature female and male mealybugs. A more opportune time could not have been selected for the collection and shipment of this material, for the great majority of the parasites were in the pupal stage and about ready to emerge. There were not, however, enough unparasitized mealybugs to provide for the parasites that had been propagated in the laboratory. Because of more or less continuous rains during the week, the material taken from the mulberry tree was sodden with water, and consequently before packing it was spread out to dry in a warm room. The heat of the drying process caused thousands of *Tetracnemus pretiosus* to issue. This material was packed in insect-tight boxes and brought to California in the vegetable room of the steamship Tahiti at a temperature ranging around 38° F. After being unpacked in the quarantine room at Riverside, many thousands of *Tetracnemus* were obtained from the mulberry-tree material.

The horticultural quarantine laws of Australia prohibit the entry of American potatoes into New South Wales and the laws of California prohibit the entry of Australian potatoes. Since the plan was to transport the entire stock of laboratory-grown parasites and their hosts in the ordinary type of ventilated propagating cages, the problem arose of securing host plants not prohibited entry into the United States. All parasites at work in the cages were definitely known to be primaries, and as their host, *Pseudococcus gahani,* was already a pest in California, the entry of these would be permitted. The Australian potatoes on which the mealybugs were growing were prohibited, as were lemons and other hosts of Australian origin.

In order to comply with the quarantine regulations, American-grown potatoes were secured. The steamers of the Matson Line carry American potatoes in their stores of food on the voyage between San Francisco and Sydney. Since the seasons in the northern and southern hemispheres are opposite, potatoes grown in the northern hemisphere sprout readily during December, January, and February, when the southern-grown potatoes are tco immature to sprout. The necessity of securing potatoes of American origin was explained to Mr. Butler, Chief Horticultural Quarantine Officer, New South Wales. He very generously cooperated, as did the officials of the Matson Navigation Company, so that two sacks of American-grown potatoes were ob-

tained from the American steamer Sierra. These were permitted entry into Sydney under certain quarantine restrictions, which specified that they were for scientific use, must be kept in cages in the laboratory, and eventually destroyed or shipped out of the country.

The potatoes obtained from the steamship Sierra were selected for their freedom from disease and for the size of their sprouts. Many of them had already produced sprouts 3 to 4 inches long. In preparation for the voyage to California it was necessary to transfer the mealybugs from the sprouts of Australian-grown potatoes to those of American origin.

The identity of the mealybug that was injurious to deciduous fruit trees in New Zealand under the name of *Pseudococcus comstocki* (Kuw.) was questioned. The doubt concerning the identity of the mealybug pest in New Zealand arose because prior to our determination of the Australian specimens as *Pseudococcus gahani* they had been confused with *P. comstocki* (Kuw.). The microscopic characters then used by taxonomists to separate *P. gahani* and *P. comstocki* are not reliable, although in life the two species are reputed to be very unlike and easily separated by the differences of the waxy filaments.

If, as anticipated, the pest in New Zealand should prove to be *P. gahani*, and not *P. comstocki* as recorded, then by going via New Zealand on the steamship Tahiti there would be a chance to obtain an additional supply of mealybugs to supplement the Australian stock on hand. This possibility was explained by letter to D. Miller, Government Entomologist, New Zealand. He was on the dock when the Tahiti arrived at Wellington, February 27, 1928. Because of heavy rains it was impossible to visit deciduous orchards, so several greenhouses were visited where grapes were being grown. In one of these a very heavy infestation of *P. gahani* was discovered and in an adjoining apple orchard a severe infestation occurred. A box of grapes infested with these mealybugs was obtained.

During the three weeks en route to San Francisco on the steamship Tahiti, the procedure was practically the same as that followed while in Sydney. A vacant hospital room, with light and ventilation, was used as an insectary, and the material was tended daily. The potatoes remained in good condition and mealybugs were available in abundance after the material was obtained at Wellington.

At San Francisco it was necessary to remove and destroy all the grapes before the material was shipped to Riverside, because of the quarantine against hosts of the Mediterranean fruit fly.

DESCRIPTION AND BIOLOGY OF COCCOPHAGUS GURNEYI

The first specimen of *Coccophagus gurneyi* Compere was captured alive on an oleander leaf in the Sydney Botanical Garden, and at the time was considered merely a specimen of scientific interest (Compere, 1928). As a general rule, the parasite collector is not interested in specimens collected indiscriminately but is primarily concerned with reared specimens having definite host records. However, because of the collector's particular interest in all species of *Coccophagus* and their host relations, this specimen was kept alive, without any expectation that it would eventually prove to be one of the most valuable parasites transported from one country to another.

Curiosity prompted a few experiments to discover the host of the female *Coccophagus*. The oleander bush on which the parasite was captured was infested with *Aphis nerii* Fons., *Saissetia oleae* (Bern.), *Pseudococcus longispinus* (Targ.), and an undetermined lecanine scale, possibly *S. persimile* (Newst.). It was supposed that one of the lecanine scales on the oleander was the host of this parasite, for prior to the discovery of this species there were only two records (both questionable) of a *Coccophagus* having been reared from anything but lecanine scales. Samples of the two scales were placed in the vial with the *Coccophagus,* but she was not interested in them. The next test was made with oleander aphis, with similar negative results. In the final test two specimens of long-tailed mealybug, *P. longispinus,* were placed in the vial with the parasite. Immediately upon sensing a mealybug, the *Coccophagus* inserted her ovipositor. Since the long-tailed mealybug is not of economic importance in California and the *Coccophagus* was thought to be nothing more than a novelty because of its unusual host, no further special care was given her and she died the next day. The mealybug in which she inserted her ovipositor was subsequently mislaid and lost. At the time it was not known that occasional specimens of *P. gahani* were generally scattered on oleanders throughout the Botanical Garden and the testing of the parasite on them was not considered; for at that time only five specimens of *P. gahani* were available and these were being carefully preserved so that they would propagate.

The second specimen of *Coccophagus gurneyi* obtained was a female. She was dead when discovered and had issued from a long-tailed mealybug that had been isolated in a small vial.

The third specimen of *Coccophagus gurneyi* was also a female. She was reared on November 24, 1927, from a long-tailed mealybug collected under a piece of loose bark in the citrus orchard of Fred Chilton, at Warrawee, about 10 miles from Sydney. This specimen was given special care, since it was then known definitely that *Coccophagus* not only oviposited in mealybugs but also developed in them, and at this date enough citrophilus mealybugs had been propagated so that a few specimens could be spared for experimentation. This *Coccophagus* was confined in a vial with a half-grown specimen of *Pseudococcus gahani*. She immediately oviposited. The next day the mealybug was dissected and eggs of characteristic coccophagine shape and size were observed. On three subsequent days different lots of citrophilus mealybugs were exposed to attack by the *Coccophagus* and after being oviposited in they were transferred to a potato sprout where they were allowed to develop. On November 28, the *Coccophagus* was liberated in a jar with a good supply of citrophilus mealybugs. On following days dissections were made of the mealybugs exposed to parasitism. These dissections showed that the *Coccophagus* eggs had hatched and that the young larvae were growing. It was definitely known that the female *Coccophagus* was a virgin and that probably her progeny would be males, so a sharp lookout was kept for the appearance of a male in order to fertilize her and thus insure female offspring. On December 18, 1927, a male was captured in a cage which was supposed to contain citrophilus mealybugs free from parasites. The original female, reared on November 24, was still alive. She was confined for a few minutes in a vial with the male. Mating promptly occurred and the fertilized female was returned to a special jar and provided with new hosts.

The progress of the individual specimens was not observed after the last week in December, for, unknowingly, *Coccophagus* in the egg and larval stages were introduced into the cages containing presumably parasite-free mealybugs. In view of what eventually happened, it is certain that some *Coccophagus* must have issued and oviposited without being noticed. In January, hundreds of *Coccophagus* emerged unexpectedly. In February, *Coccophagus* began to issue in overwhelming numbers and it became necessary to destroy a surplus each day in order to preserve a balance between the stock of mealybugs and parasites. The surprising rapidity with which *Coccophagus* and the other natural enemies developed made it advisable to rush the entire lot of material to California, where unlimited quantities of host insects were available.

During March, April, and May, 1928, *Coccophagus* were propagated in the insectary of the Citrus Experiment Station at Riverside. The first colonies were liberated in the citrophilus-mealybug-infested areas of southern California during June, and at the same time colonies were offered to the operators of the various local insectaries which were engaged in the mass production of *Cryptolaemus*. On July 24, the first recovery was made, when specimens of *C. gurneyi* were reared from citrophilus mealybugs infesting a sapota tree located in the city of Whittier. After the date when the first recovery was made, specimens were taken in rapidly increasing numbers from all localities where colonies had been liberated and test rearings made. Within a year the species was thoroughly established throughout the greater part of the infested area of southern California and in parts of the San Francisco Bay region. In July, 1929, the propagation of *Coccophagus* was discontinued by the Citrus Experiment Station, as it was considered that the species was thoroughly established.

The Adult.—The female of *Coccophagus gurneyi* can be distinguished by coloration from all other described species of *Coccophagus* with one exception; the body is black, with a conspicuous band of yellow across the base of the abdomen (fig. 2). The body of the male is entirely black and it cannot be so easily recognized as the female (Compere, 1929).

Oviposition.—The adults are slow and deliberate in their movements. When ovipositing, the females are not easily disturbed and they will persist in their efforts unless they are prodded or forcibly removed. Mating and egg laying begin soon after emergence. Adults are long-lived; females have been frequently observed ovipositing more than three weeks after they were confined in cages.

In a series of experiments to obtain egg-laying records, some adults lived for 27 days when confined in a small vial. During their confinement these specimens were given drops of water and sugar and provided with a fresh mealybug each day, which not only served as a host but supplied food in the form of honeydew. In one set of tests, 67 eggs were deposited by a single female within the first 48 hours. This female did not again oviposit until 3 days later, when 17 eggs were laid. Two days later she laid 40 eggs. One female lived for 2 weeks without depositing any eggs. Others produced 1 or 2 eggs daily over a period of 15 days. In the majority of cases, oviposition extended over a period of 15 days when the parasites were confined in small vials. After oviposition ceased the parasites continued to live. One female lived for 27 days and deposited a total of 45 eggs, of which 23 were laid on the first day.

These preliminary data in regard to fecundity are too meager to permit even a rough estimate of the normal reproductive capacity, and they also suggest the probability that experiments with specimens closely confined are not a reliable index of what happens under natural conditions. It is definitely known, from field observations and cage work, that under normal conditions the adults are long-lived and oviposit over a considerable period of time. In the general account, mention was made of a female that issued on November 24, 1927, and was under observation until December 18, when she was mated and then allowed to resume ovipositing.

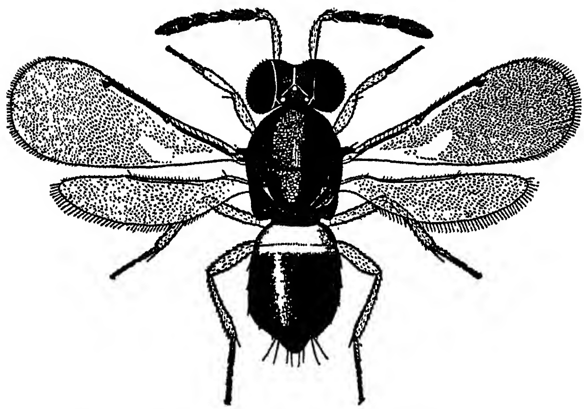

Fig. 2. *Coccophagus gurneyi* Compere, female.

Citrophilus mealybugs of both sexes and of all stages are attacked by this parasite. In the cages it is not uncommon to see *Coccophagus* ovipositing in the winged males which swarm on the cloth during certain hours of the day. It seems unlikely that *Coccophagus* can develop to maturity in the adult males, but this supposition has not been proved. The immature males, or pupae, contain sufficient food to nourish the *Coccophagus* to maturity. Many of the *Coccophagus* produced in the insectaries for field colonization developed on immature male mealybugs. If the cottony masses which are found in the cages are examined, it is seen that they contain large numbers of male pupae, and parasitized specimens can be detected.

Normally only one egg is deposited in a mealybug. Females do not distinguish between parasitized and unparasitized hosts and, consequently, a single host will often be attacked by more than one parasite. Repeated ovipositions occur in one mealybug when there are too few hosts. This applies to specimens under natural as well as under insectary conditions, as is shown by dissections. Mealybugs

from a locality where the adult parasites are abundant often contain 5 or 6 eggs, the supernumerary ones of which are in the process of being destroyed by the so-called 'phagocytic action.'

Occasionally *Coccophagus* eggs are found in the host's visceral organs or in the ovarian eggs, but generally they are deposited in the body cavity and float out into the solution in which the host is dissected.

When about to oviposit, the female approaches the host in the manner usual to many parasites. A short preliminary examination is made by sensing the mealybug with the ends of the antennae. If satisfied with the preliminary inspection, she stands over the site selected for insertion and flexes the end of the abdomen downward and forward to bring the apex in contact with the host. The tip of the ovipositor is fixed against the derm of the host and then the abdomen is returned to the normal horizontal position leaving the protruded ovipositor extending perpendicularly to the host. Several seconds to three-quarters of a minute, according to the size of the host and the toughness of the derm, elapse while the derm is being pierced. While drilling through the derm the parasite remains motionless, in an upright position, antennae elbowed and hanging downward, the wings in repose. As the tip of the ovipositor penetrates the derm the abdomen is lowered, forcing the exserted ovipositor its full length into the mealybug. Usually the egg is not deposited immediately, but only after the parasite partly retracts and inserts the ovipositor in probing movements. This probing is significant; for if the mealybug is already inhabited the occupant is usually detected by the *Coccophagus,* which then responds differently, according to the type of larva encountered. This is further discussed under the heading, "Biological Interrelations of Host and Parasites."

The Egg.—The egg is of the usual coccophagine shape, elongate, slightly arcuate, and widened anteriorly, as shown in figure 3*A*. The micropyle is usually conspicuous and rather large, being visible in the ovarian eggs as well as in those newly laid. The micropylar end is sometimes folded and flattened like a miniature cap or imperfectly formed pedicel. The chorion is smooth and transparent, clearly revealing the contents which, in the newly laid egg, are opaque, white, and homogeneous. The newly laid egg measures about 0.16 mm long by 0.04 mm wide. The egg enlarges as the embryo develops. Before hatching, the fully formed larva is clearly visible within the transparent chorion. At summer temperature, 27 days elapse from the egg to the adult stage, and the eggs hatch in approximately 4 days.

The Larva.—The newly hatched larvae are not unlike those seen while enclosed in the chorion. At hatching, the alimentary canal contains some material which was obtained while in the egg. After the larva begins to feed, reddish particles appear in the alimentary tract. The first-stage larva has twelve definable body segments, exclusive of the head and tail. The tail is not so attenuated and slender as in some species of *Coccophagus,* and in certain positions it appears much like a thirteenth body segment. The mandibles are small and not easily seen. A drawing of a first instar larva is shown in figure 3*C*.

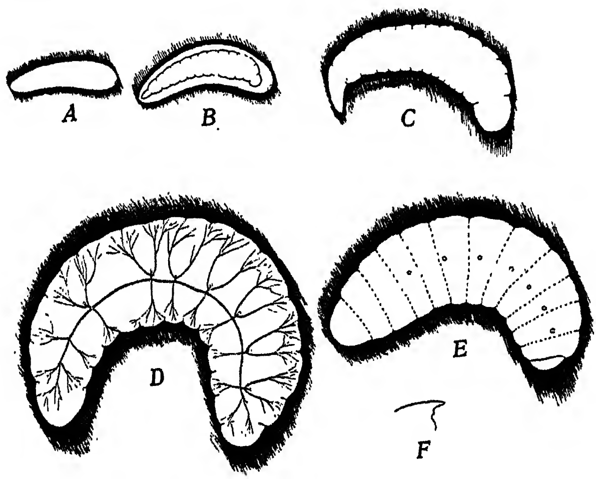

Fig. 3. *Coccophagus gurneyi* Compere. *A,* newly laid egg; *B,* egg just before hatching; *C,* larva several days after hatching; *D,* mature larva showing tracheal system; *E,* larva after voiding meconium; *F,* mandible of mature larva.

No effort was made to count the number of molts and instars. Figure 3*C–E* shows three larval instars.

In only one respect does the larva seem unusual. When fully grown it has only seven pairs of spiracles, while in all the other known species nine pairs of spiracles are usually present. Open spiracles do not appear until the last instar. In the penultimate instar the tracheal system is well developed and seven closed spiracular branches are present.

The fully mature larva completely fills the body of a small or partly grown mealybug, but in a mature mealybug the body is not entirely occupied.

At summer temperatures, on about the seventeenth day after the eggs are laid, the larvae void the contents of the alimentary tract,

pupate, and several days later the pupae begin to blacken. During the summer months about 27 days elapse between egg deposition and the emergence of the adults.

Appearance of Parasitized Mealybugs.—The mummified bodies of mealybugs destroyed by *Coccophagus* are usually grayish or fuscous owing to the dark-colored pupal remains which underlie the hardened parchment-like derm of the host. Specimens occasionally retain the waxy filaments and powder after mummification so that the characteristic dark coloration is obscured. The exit holes through which the parasites issue are usually located dorsally near the posterior end of the host. The pupa lies ventral side downward with its head end at the posterior end of the mealybug. The mummified mealybugs are most abundant in places of concealment, such as under trap bands, in dried leaves, or under loose bark. It seems evident that parasitism causes the mealybugs to desert the exposed feeding areas prematurely in search of a place of concealment.

Destruction of Supernumerary Eggs and Larvae.—As a general rule only one *Coccophagus* matures in a single host. This was thought to be an invariable rule, and it has been so in the case of thousands of specimens examined. Helen Perry, a laboratory assistant who was engaged in making dissections to obtain records concerning the percentage of parasitism in the orchards, first called attention to several specimens of citrophilus mealybugs containing more than one larva. In one particular mealybug, 5 perfectly formed pupae of *Coccophagus gurneyi* were discovered. A possible explanation of this rare situation is that these *Coccophagus* developed as accidental secondaries on *Pseudaphycus angelicus* (How.), a species with gregarious habits that very rarely parasitizes the citrophilus mealybugs in California.

If more than one *Coccophagus* oviposits in a mealybug, or if a single parasite deposits more than one egg in an individual host, none may develop, or only one of these eggs may reach maturity. Supernumerary eggs and larvae are destroyed by some process which appears very similar to the so-called 'phagocytic action' that destroys *Coccophagus* eggs in an immune host such as *Pseudococcus citri* (Risso). Possibly, as believed by some entomologists, the phagocytic action is a secondary process acting upon organisms that have been killed by some more obscure, defensive host reaction. Regardless of the nature of this defensive reaction, it not only kills supernumerary eggs and larvae but it also inhibits the development of the surviving parasite. This is shown by the size of solitary larvae when compared with those that survive in competition. In extreme cases when 10 to 20 or more eggs are deposited by *Coccophagus* in a single mealybug,

all the eggs and larvae as well as the host succumb. In cages where excessive parasitism occurs, it is not uncommon to see hundreds of shrunken, dead citrophilus mealybugs. If dissected, these dead mealybugs will be found to contain numerous dead and dying *Coccophagus* eggs and small larvae. In contrast *P. citri,* a perfectly immune host, will successfully destroy as many as 54 *Coccophagus* eggs without appreciable injury.

Phagocytosis is characterized by the presence of reddish cells which congregate around the eggs or larvae. As the process continues, the cells contract and harden and the entire mass comes to rest in the form of a small black pellet. These pellets usually lodge just beneath the derm of the host, through which they are readily seen.

DESCRIPTION AND BIOLOGY OF TETRACNEMUS PRETIOSUS

The first specimen of *Tetracnemus pretiosus* Timberlake that was reared from mealybugs was obtained from material collected under a piece of loose bark in débris in the citrus orchard of Fred Chilton, at Warrawee, New South Wales, November 19, 1927 (Smith and Compere, 1928). This lone female *Tetracnemus* issued November 27, 1927, and was captured and placed in a vial with some specimens of *Pseudococcus gahani.* She readily oviposited in them. She was next liberated in a jar with a plentiful supply of mealybugs in which to oviposit. On December 7, an inspection of the jar was made and the adult *Tetracnemus* was found dead. At this time she was recognized as being specifically the same as a specimen (the host of which was unknown) that had been collected at random in the Sydney Botanical Garden some time previously.

During January, male *Tetracnemus* began to issue from this jar. On subsequent days a few females were noted. The latter, and no doubt some of the males, were unknowingly introduced into the stock when in the egg or larval stage, concealed within their hosts.

Tetracnemus were reared by thousands on February 19, 1928, when a large quantity of *Pseudococcus gahani* was brought into the laboratory. This material was obtained from the infested mulberry tree that was discovered January 13, 1928, on Bulwara Road, Sydney.

The parasites readily reproduced at the Riverside insectary, and colonies of *Tetracnemus pretiosus* were supplied to the Orange and Los Angeles county insectaries on April 23 and 24, 1928, and at the same time colonies were liberated in the field. The first recovery was

made on August 15, 1928, when *Tetracnemus,* in company with *Diplosis* sp. and *Coccophagus gurneyi,* were reared from *Pseudococcus gahani* infesting a sapota tree located in Whittier. At about the same date, D. W. Tubbs reported rearing *Tetracnemus* from mealybugs collected in Orange County. In every locality where the species was colonized it quickly became established.

The Adult.—The adult female *Tetracnemus* (fig. 4) cannot be briefly described in a way that will permit its ready identification. The original description by Timberlake should be consulted for the

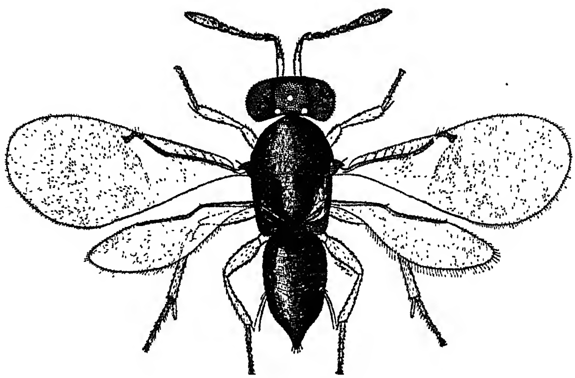

Fig. 4. *Tetracnemus pretiosus* Timberlake, female.

determination of the adults (Timberlake, 1929). The male, which has branched antennae, is more easily recognized than is the female.

The life history of this valuable and interesting species has not been fully worked out.

Oviposition.—The remarkable oviposition habit of this species was demonstrated in Sydney on February 21, 1928, when hundreds of *Tetracnemus* escaped from the material being prepared for shipment to California. Citrophilus mealybugs were hatching at the same time and some of them escaped before the shipping boxes were closed. The *Tetracnemus* were attracted to the newly hatched mealybugs crawling on the boxes and were energetically ovipositing in them, one after another, with hardly a pause between ovipositions.

Female *Tetracnemus* readily, if not preferably, attack very small mealybugs. The long, slender ovipositor is extended from the apex of the abdomen and the parasite faces away from the victim. The ovipositor is plunged into the body of the mealybug on the first thrust and the egg quickly deposited. No particular spot on the host is selected as the site for the insertion of the ovipositor. It may be

thrust into the anterior end of the mealybug just as often as into the posterior end. The evidence indicates that most of the eggs are deposited within the first few days after emergence and that the adults are short-lived.

Temperamentally *Tetracnemus* is very different from *Coccophagus gurneyi;* the former is more active while the latter is slow, deliberate, and apparently more methodical in its habits.

The Egg.—Considerable difficulty was experienced in locating the eggs of *Tetracnemus* after they were deposited in a mealybug. A few eggs were recovered after small mealybugs were exposed to attack and then dissected. Newly deposited eggs are exceedingly small and are not usually detected when the ordinary low powers of a binocular miscroscope are used. They measure about 0.03 mm in length, inclusive of the pedicel, as indicated in figure 5*B*.

The Larva.—The illustrations of the egg and larval stages are not drawn to scale, but the sizes are indicated by actual measurements in millimeters. Presumably five, or possibly six, larval instars were observed. The notes and drawings illustrating the life history of *Tetracnemus* were obtained by a study of specimens dissected from mealybugs at intervals during the development of a single generation of parasites. This life history study should be verified and amplified, for there is uncertainty regarding several very interesting stages.

Pseudococcus gahani in a cage were exposed to the attack of *Tetracnemus* on August 9, 1928, and then isolated. By August 21, the more advanced larvae had consumed the entire body contents of the mealybugs and were casting their meconia. The mature larva orients itself parallel to the long axis of the host and expels the meconium in one end, where it appears as a 'black cap.' The mealybugs remain alive and active until the parasitic larvae are almost fully grown. A single specimen possibly representing an instar not illustrated, was discovered. The specimen had cast its meconium and the mandibles were distinctly serrated at the apex with three minute, acute teeth. The generation of parasites that started August 9, began to issue September 1, 1928, a period of 23 days elapsing from the deposition of the egg to the emergence of the adults.

The incubation period was not determined. On the fourth day the specimens had increased to about eight times their original size and had assumed the shape and appearance of larvae, although still enclosed in the transparent chorion. Presumably these 4-day-old specimens were embryos within the eggs and the chorion had enlarged and closely adhered to the developing larvae. Figure 5*C* and *D*

represents this stage. The 5-day-old specimen shown in figure 5*E* does not show an increase in size compared to those noted the previous day, and it is still enveloped by the closely adhering, form-fitting chorion, but the embryo or larva shows considerable development.[5] Specimens dissected from mealybugs on the seventh day were free

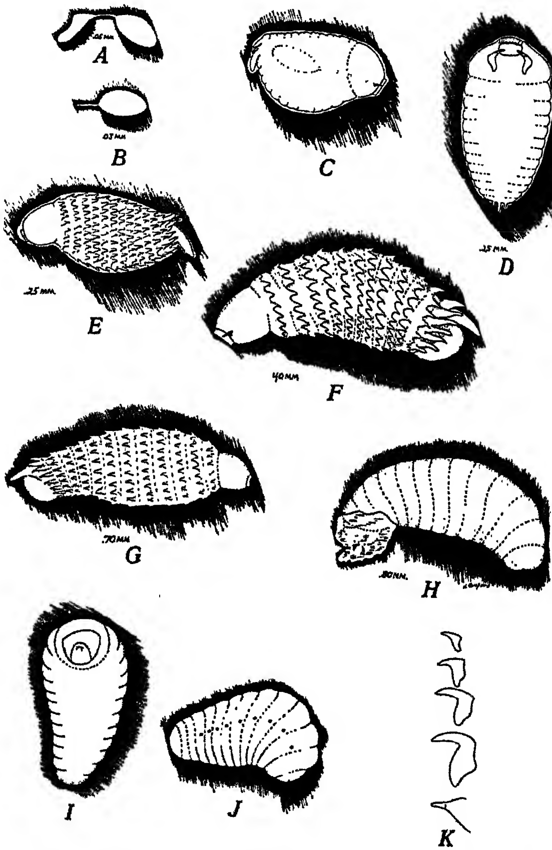

Fig. 5. *Tetracnemus pretiosus* Timberlake. *A*, ovarian egg; *B*, newly laid egg; *C–D*, larva just before hatching, lateral and ventral views; *E–J*, larval instars; *K*, series of larval mandibles. (The series of mandibles are drawn to scale, the other figures are not.)

of the enveloping chorion and had grown to one and one-half times the size of the larvae that were observed on the fifth day. The 7-day-old specimens showed the tracheal trunks beginning to develop in the

[5] It should be noted here that the eggs laid by this adult *Tetracnemus* were deposited during a period of 12 hours, so that a discrepancy may appear when the larvae are identified by their age as counted in days.

region of the third segment; the tubercle-like projections and the head were relatively smaller in proportion to the size of the body; while a bulging posterior protrusion had developed. Larvae 8 days old, counting from the deposition of the egg, had increased to almost twice the size of the specimens noted on the seventh day; the tracheal system was similar but the head and projections were relatively smaller.

A 9-day-old larva is shown in figure 5*H*. The last molt skin is adhering to the posterior end, the tubercles characteristic of the preceding instars have disappeared, the head has undergone a marked change, and the mandibles and mouth parts are radically altered and reduced. Larvae removed from mealybugs on the twelfth day were about ready to pupate and did not possess any distinctive characteristics which would readily distinguish them from the larvae of many other encyrtid parasites. In this stage nine pairs of open spiracles appear and the segmentation is distinct. The fifth and largest pair of mandibles, as shown in figure 5*K*, is associated with this instar. It is possible that the largest mandibles figured possess minute apical teeth, but if so, they were concealed by their position.

The series of mandibles is drawn to scale, and they are associated with the specimens representing the different instars as figured.

So far as observed, only one larva develops in a single mealybug. The process by which supernumerary eggs and larvae are destroyed was not noted.

Appearance of Parasitized Mealybugs.—It is not always easy to distinguish between mealybugs destroyed by *Tetracnemus* and those destroyed by *Coccophagus*. Generally, however, the host remains of *Tetracnemus* are characterized by the paler color of the 'mummy,' and by the more uniform and regular appearance of the meconial discharge which appears at one end, giving the 'black cap' appearance.

The Present Status of Tetracnemus in California.—In the areas where *Tetracnemus* was established before *Coccophagus*, it rapidly became very abundant and indicated that it was able to bring an infestation under control. With few exceptions the range of *Tetracnemus* was soon overlapped by that of *Coccophagus* and the latter species became dominant. Occasionally limited areas were found where *Tetracnemus* was not replaced and effectively controlled the mealybugs. *Tetracnemus* is now generally present throughout the infested area but in smaller numbers than is *Coccophagus*. There is evidence indicating that during the past two seasons (1930 and 1931) the *Tetracnemus* population gained relative to that of *Coccophagus* during the summer months, and lost during the winter months.

HABITS OF ANUSOIDEA COMPEREI

Anusoidea comperei Timberlake is presumably a primary parasite of the citrophilus mealybug, *Pseudococcus gahani* Green. For a discussion of the adult characters of this species the reader is referred to the original description (Timberlake, 1929).

On December 4, 1927, in Sydney, a single female specimen of *Anusoidea* issued from an undetermined mealybug. This mealybug was segregated in a small vial; for it was obviously parasitized at the time of collection. Soon after the parasite issued she was tested in a vial with samples of *Pseudococcus gahani.* She readily oviposited in them. After being allowed to oviposit for a short period, this female was isolated. Since she was a virgin it was anticipated that her progeny would be males. This proved to be the case, for male specimens appeared January 2 to 7, 1928. During the interval of waiting for the appearance of male specimens, the female was regularly fed and kept in a cool place. She was alive in January when her male offspring issued and she was then mated to them. After being fertilized she was allowed to resume oviposition. Both male and female progeny resulted from this union, and began to issue February 10. When mating couples were noticed they were captured and placed in other cages. Unfortunately, practically the entire third generation of *Anusoidea* was consigned to cages which eventually developed a large number of *Coccophagus.* Only a few male specimens of a fourth generation matured. The loss of *Anusoidea* was possibly due to replacement while in the larval stage by *Coccophagus.* It was not introduced into California.

HABITS OF MIDAS PYGMAEUS

It is not known whether *Midas pygmaeus* Blackburn (fig. 6) is permanently established in California. During 1929, prior to the general control of the mealybug by parasites, occasional specimens of *M. pygmaeus* were taken from under the burlap bands in areas where the colonizations were made. *Midas* was imported into California at the same time as were the internal parasites of *Pseudococcus gahani.* It was introduced with the expectation that it would be of considerable value, for in New South Wales it occurred generally wherever *P. gahani* was found.

The larvae of *Midas pygmaeus* (fig. 7) were first collected in the Sydney Botanical Garden, where specimens were discovered in cracks and places of concealment, feeding on the eggs of the few citrophilus mealybugs that reached maturity. *Midas* was never taken working on either *Pseudococcus citri* or on *P. maritimus,* although several severe infestations occurred in proximity to the places where *Midas* was collected. Very often the presence of a few citrophilus mealybugs on *Grevillea, Pittosporum,* or *Nerium* was indicated by the presence of a stray *Midas* larva.

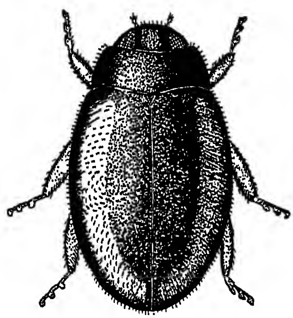

Fig. 6. *Midas pygmaeus* Blackburn, adult.

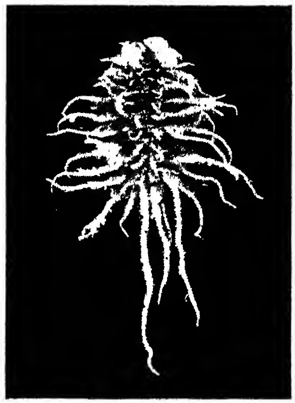

Fig. 7. *Midas pygmaeus* Blackburn, larva.

One peculiar trait of *Midas* is its habit of shunning the light and remaining concealed. It is not easy to collect adults propagated in cages because they remain in the cracks of the soil or work down around the potato tubers.

An interesting reference to *Midas pygmaeus* was made by Albert Koebele in 1893, when he wrote:

> This insect was bred from a white, tufty larva found upon orange at Paramatta, New South Wales. It was also collected in considerable numbers at Toowoomba, Queensland, upon the same tree. Became quite abundant at Sydney during March upon oleander and *Pittosporum* infested with a species of *Dactylopius* upon which they appear to feed. Was also found at Mulgoa, New South Wales, upon eucalyptus. Many specimens sent to California.

In the unpublished letters of George Compere there are records showing that he made shipments of *Midas* to California from New South Wales.

A stock of *Midas pygmaeus* is still maintained in some of the local insectaries and occasional colonizations are being made.

DESCRIPTION AND BIOLOGY OF DIPLOSIS SP.

This species of *Diplosis* has not been determined. It is possibly the same as the species discovered by Albert Koebele in Sydney and reported by him in 1893 under the name *D. koebelei* Skuse MS.

Diplosis sp. was reared from various mealybugs collected in the Botanical Garden and in the vicinity of Sydney during the period from November to February, 1927. The species readily propagated on *Pseudococcus gahani* when placed in the cages, and multiplied so rapidly that it was necessary to destroy the excess of adults continually in order to preserve the stocks of mealybugs.

Diplosis was imported into California in March, 1928, with the other natural enemies of *Pseudococcus gahani* from Sydney. Colonies were placed in the orchards during the spring of 1928 and the first recovery of adults was made August 15, 1928. As in the case of the other natural enemies of citrophilus mealybugs, the Orange County and Los Angeles County insectaries took the lead in the mass production and distribution. *Diplosis* rapidly established itself where it was colonized. Occasional *Diplosis* larvae were found on mealybugs submitted for examination during the 1928–29 season. Fewer recoveries were made during the 1929–30 season. This species is thought to be permanently established in California, but its influence is negligible.

The life history of *Diplosis* was worked out by Mumtaz Arif, a graduate student located at the Citrus Experiment Station during the summer of 1929. His studies have not been published.

The following data were obtained while engaged in the work of propagating the species; no special effort was made to obtain life-history notes.

The Adult.—The adults are not easily handled in confinement. When placed in small containers they injure themselves in their continued efforts to escape. In cages or large containers their behavior is more nearly normal. In the small containers individual specimens lived not more than 4 days. The adult life under cage conditions was not ascertained. The peculiar habit of congregating on spider webs, which is commonly seen with many cecidomyids, is highly developed in this species. In the cages it is a common sight to see dozens of specimens swinging to and fro in unison on a single web.

The Larva.—If taken associated with *Pseudococcus gahani*, the larvae of *Diplosis* can be readily recognized since the only other maggots likely to be found associated with this insect are those of *Leucopis*. *Leucopis* has grayish larvae, while those of *Diplosis* are orange with the digestive tract appearing as a dark longitudinal streak. The larval period averages about 7 days during the summer months. Larvae readily feed upon all stages of mealybugs. They pierce the host and suck out the fluid contents.

The Pupa.—Pupation usually takes place about 9 days after the eggs are laid. Pupae are usually found in old egg masses or in proximity to the infestations. The pupal stage lasts about 4 days on the average.

CHRYSOPA RAMBURI AS AN ENEMY OF PSEUDOCOCCUS GAHANI

The larvae of *Chrysopa ramburi* Cameron were especially conspicuous on various coccid-infested shrubs in the Sydney Botanical Garden. The larvae are trash carriers and are very active, running along the limbs and twigs with their backs matted with the remains of coccids and other débris. This lacewing reproduced readily in the cages on *Pseudococcus gahani*. A colony was brought to California and living specimens were supplied to several insectaries. It is doubtful if this species is established in California.

BIOLOGICAL INTERRELATIONS OF HOST AND PARASITES

Timberlake (1913) first recorded the fact that under certain conditions *Coccophagus lecanii* (Fitch) is able to develop on other scale parasites. Although he stated very plainly that this type of parasitism was accidental and that true primary parasitism was the rule, the statement that this valuable species occasionally developed hyperparasitically was quoted by certain authors without being qualified, and the species was, therefore, recorded as a hyperparasite. The genus *Coccophagus* includes some of the most valuable scale-destroying parasites. It is possible that most of the species which have solitary larvae are capable of development on other primary parasites, or upon individuals of their own species under certain conditions. This is the case with *C. gurneyi*. The existence of such a habit is of no economic significance, since there is no selection by the

parasite of parasitized hosts in preference to unparasitized ones. It is, however, of considerable interest biologically.

If an ovipositing *Coccophagus* attacks a mealybug which is already inhabited by a larva, the egg originally intended for the mealybug will be deposited either within or upon the first occupant. Timberlake recorded the remarkable fact that when *C. lecanii* develops hyperparasitically its habits undergo a radical change and it develops as an ectoparasite on the first inhabitant, when both are within the scale. It has been found that *C. gurneyi* is capable of both ecto and endoparasitic development in relation to the first inhabitant. The first evidence of this ability to develop either way was observed when both *C. gurneyi* and *Tetracnemus pretiosus* were being propagated in the same cages. When these mixed populations occurred, *Coccophagus* was always dominant and in time completely eliminated *Tetracnemus.*

Tetracnemus deposits the major part of its eggs during the first few days and then dies. In contrast, *Coccophagus* oviposits over a period of about three weeks. Consequently, after the first week some of the ovipositing *Coccophagus* chance upon some of the mealybugs inhabited by *Tetracnemus* larvae. When an ovipositing *Coccophagus* encounters a *Tetracnemus* larva, it deposits the egg originally intended for the mealybug within the body of the *Tetracnemus.* Many of the mealybugs which were exposed to excessive numbers of *Coccophagus* and *Tetracnemus* in the same cage were found to contain *Tetracnemus* larvae having from one to six or more *Coccophagus* eggs inside them. Frequently the *Tetracnemus* larvae were distended by being completely filled with *Coccophagus* eggs. In many cases in addition to the eggs found in the *Tetracnemus* larvae, parasite eggs were found free in the mealybug's body. Although *Coccophagus* does not discriminate between parasitized and unparasitized mealybugs, it seems evident that oviposition occurs in the primary larva in preference to the mealybug itself whenever a previously parasitized host is attacked. Almost invariably when a primary occupant is met by the probing ovipositor of a *Coccophagus,* the egg is placed either upon or within it. However, if the first occupant is not detected by *Coccophagus,* then the egg is deposited in the body of the mealybug itself.

When this habit of *Coccophagus* was discovered, it was thought that possibly it could develop on a parasite within a mealybug which was immune to parasitism by *Coccophagus.* For this experiment *Leptomastidea abnormis* (Girault) inhabiting *Pseudococcus citri* were

used.[6] A remarkable thing occurred. If the *Leptomastidea* larvae were small and were not detected by the ovipositing *Coccophagus,* the eggs were deposited free in the mealybug's body where they were destroyed by the physiological processes which render this species of mealybug immune to parasitism by *Coccophagus.* However, if the ovipositing *Coccophagus* chanced upon the larva of a *Leptomastidea*, it deposited its eggs upon the body of this primary inhabitant and became ectoparasitic in relation to it. On dissection the *Coccophagus* eggs were found adhering to the derm of the *Leptomastidea* larvae. The ultimate fate of *Coccophagus* growing on *Leptomastidea* is determined by the condition of the mealybug. If the mealybug has not been seriously weakened by the *Leptomastidea,* the normal defensive reaction of the mealybug destroys the *Coccophagus* eggs while they are in place on the body of the *Leptomastidea;* but, if the *Leptomastidea* has consumed the fluids of the mealybug, the protective reaction of the mealybug is destroyed, and the *Leptomastidea* has rendered itself vulnerable to *Coccophagus.*

Under certain conditions *Coccophagus gurneyi* is capable of destroying mature *Leptomastidea abnormis* larvae and prepupae, but it appears to be incapable of developing to maturity on these hosts. Out of hundreds of trials, not a single *Coccophagus* successfully completed its development on *Leptomastidea.* Under suitable conditions the *Coccophagus* eggs will hatch and the larvae will destroy *Leptomastidea,* but according to our observations *Coccophagus* always fails to reach maturity, and all three insects die. The influence of *Coccophagus* on *Leptomastidea* is believed to be negligible.

Coccophagus reacts to finding its own larva in a mealybug much the same as it does when accidentally encountering a *Tetracnemus* larva, that is, it deposits its egg within the first inhabitant, which is eventually consumed by the larva that hatches last.

When developing on other parasitic larvae, *Coccophagus* larvae grow much faster than they do when developing on the mealybugs themselves. This has been observed in the case of other species which have this habit. It is suggested that the more rapid development is a result of the use of food which has been already elaborated by a prior inhabitant of the host.

Although *Coccophagus* eggs and larvae normally develop in the body fluids of mealybugs, they are capable of development when isolated in dry containers if placed on a larva of *Leptomastidea.*

[6] *Leptomastidea* is a primary parasite of *Pseudococcus citri* (Risso), a mealybug immune to parasitism by *Coccophagus,* although the latter readily oviposits in it.

An occasional young larva of *Tetracnemus pretiosus* was found occupying a mealybug in company with a *Coccophagus* larva, but the ultimate outcome of this association was not determined. When small *Tetracnemus* and *Coccophagus* larvae are found developing within the same host it is probable that oviposition of both species was almost simultaneous. In the great majority of cases where competition was observed *Coccophagus* had oviposited after *Tetracnemus* had reached the larval stage and *Tetracnemus* then became the host of *Coccophagus.*

NUMERICAL RELATIONS OF HOST AND PARASITES

Multiple Parasitism.—It has been maintained by some entomologists (Pemberton and Willard, 1918) that it is a mistake to introduce for control purposes two or more species of entomophagous insects attacking the same stage of the host, for, according to this theory, the resulting competition reduces the total controlling effect to a point below that which would have occurred if only the more prolific species had been introduced. Regardless of the soundness of this theory, it is largely a matter of academic interest rather than of practical concern, because of the extreme difficulty, if not impossibility, of predicting how an insect will respond to a new environment, and particularly to the biotic phases of that environment.

It is well known that the potential reproductive capacity of a parasite bears no direct relation to the ability of that parasite to maintain its host at a low population density. If such a direct relation existed, the polyembryonic species would ordinarily be the most effective, when, as a matter of fact, they are probably of very little economic importance as compared to many of the monembryonic species. Reproductive potential, therefore, cannot be used as a criterion by which to make a selection of entomophagous insects for introduction into a new habitat. Neither can it be safely concluded that of two parasites attacking the same host in its native home, the one which destroys the greater percentage of hosts is the more valuable one to introduce. This assumption often proves incorrect, as it does in the present case; *Tetracnemus* was much more effective in Australia, while in California *Coccophagus* seems to be much more effective.

If the theory of the injuriousness of multiple introductions is accepted, it would be necessary to obtain a complete knowledge of the ecology of *all* the insect enemies of a particular host throughout

the world and also of the insect enemies of related hosts, before any species could be introduced. The absence of any reliable criterion by which to judge the comparative value of entomophagous insects for the purpose of biological control, makes it apparent that this theory must be largely disregarded in practical work. It is essential that the introduction of obligatory secondary parasites be avoided, but beyond this there is as yet no known criterion upon which to base further efforts at selection.

Certain theoretical objections to the hypothesis that multiple introductions are injurious have been advanced in another paper (Smith, 1929). The introduction of *Coccophagus* and *Tetracnemus* into California has provided some interesting data on the practical aspects of this question.

As has been previously mentioned, when colonies of both *Tetracnemus* and *Coccophagus* are introduced into a cage heavily stocked with citrophilus mealybugs, *Coccophagus* rapidly becomes dominant and in time completely eliminates *Tetracnemus*. Under such conditions there occurs a very high percentage of parasitism and consequently a large amount of overlapping. It is to be expected that *Tetracnemus* will disappear, since *Coccophagus* is the victor when the two meet in competition and since there are enough adult *Coccophagus* produced to parasitize *all* the mealybugs in the cage. In the field, however, the condition is very different.

If the parasites are of any great value, they will maintain the mealybug population at a low density. As the density of the host becomes reduced the percentage of parasitism must for obvious reasons become reduced also. This reduction in parasitism is naturally accompanied by a reduction in overlapping of the two species of parasites, so that the ratio

$$\frac{\text{number of hosts parasitized by } \textit{Tetracnemus}}{\text{number of hosts parasitized by } \textit{Coccophagus}}$$

varies inversely with the density of the host population. A low density of the host *must be maintained* by the parasites if they are to be of much practical value. When the density of the host is low there is a minimum of overlapping and, therefore, slight effect by one parasite on the other. Consequently, it seems entirely reasonable to conclude that these two parasites are more effective than either one alone would have been. Under such conditions each species destroys host individuals which would have escaped destruction by the other, if the parasites have slightly different habits and habitats.

In this case another advantage of two species over one is their different response to the same temperatures. *Tetracnemus* is very scarce during the winter months, while *Coccophagus* is active throughout the cold weather and develops two generations to one of its host. This winter activity of *Coccophagus,* at a time when the mealybug population is normally low, is most effective since it materially reduces the overwintering mealybugs that produce the spring brood, which caused the greatest damage in the past. During the summer, however, *Tetracnemus* causes a considerable mortality of mealybugs.

The presence of heavy ant infestations also produces a different effect on the two species. *Coccophagus* is very slow and deliberate in oviposition, whereas *Tetracnemus* oviposits very quickly. For this reason ants have more opportunity to interfere with the activities of *Coccophagus* than of *Tetracnemus.* In the infestations where the mealybugs were protected by ants, dissections have shown that *Tetracnemus* was more abundant, relative to *Coccophagus,* than was the case in the average sample from ant-free locations.

Influence of Parasites on Population Density of Mealybugs.—During the past three years many thousands of mealybugs, representing hundreds of samples from distinctly varying climatic zones in California, have been dissected for the purpose of obtaining the percentage of parasitism. This work was undertaken as a part of the process of establishing the parasites in all the infested areas, and also to obtain a better idea of their progress. It was realized that the percentage of parasitism, when not correlated with figures representing the population density of the host, is of little or no value in determining to what extent the parasites influenced host population density. It was not possible, however, with the means at our disposal, to obtain figures on population density of sufficient reliability to make them useful for this purpose.

The percentage of parasitism, as exhibited by the samples, ranged from 20 to 60 per cent, with occasional samples running much higher, and a few 100 per cent parasitized. In considering this question, however, it is necessary to bear in mind that the actual destruction of mealybugs by parasites was considerably higher than the samples indicated. Many of the mealybugs were small and would have been liable to attack for several weeks if they had not been collected. Often the specimens were collected in protected places, as between two fruits, where they were inaccessible to parasite attack. This was particularly true in places where the mealybugs had become extremely scarce. Some came from ant-infested trees.

It has frequently been observed that the presence of a parasite larva within a mealybug results in abnormal activity of the host insect. The principal effect of this kind is that it intensifies or advances the reproductive instincts of the females, causing them to migrate down the trunk of the tree when they are about half grown, when ordinarily they wait until they are full grown and ready to deposit their eggs. Bands placed about trees become packed with parasitized half-grown mealybugs, when in the absence of parasites it would have been at least two weeks before this migration took place. A result of this abnormal migration induced by parasitism is that large numbers of parasitized mealybugs leave the trees. Therefore, the samples collected for dissection have a disproportionate number of unparasitized hosts.

It is also necessary to bear in mind that there are approximately two generations of parasites to one of mealybugs, particularly in the cooler seasons. If a sample of mealybugs is dissected and only 50 per cent is found to be parasitized, this does not mean that only 50 per cent of the generation which they represent is destroyed by parasites. Before the surviving mealybugs mature, a second generation of parasites occurs and if these also destroy 50 per cent of the same generation of mealybugs, there is a total destruction of 75 per cent, although only 50 per cent of the mealybugs would contain parasites at any one dissection. It is obvious, therefore, that the actual percentage of destruction of mealybugs by parasites must in most cases be far higher than the dissections have indicated.

The percentage of parasitism, however, taken by itself, is of relatively little value in judging the effect of a parasite on the population density of its host. The general tendency to overestimate the value of such figures for this purpose must be guarded against. Insect enemies are only one of many causes of mortality of a plant-feeding insect, and many of these factors are interdependent in their action. Therefore, it is extremely difficult to determine how the presence or absence of any one factor, such as parasites, will influence the population of the organism against which it operates. A high percentage of destruction by one factor can be important or unimportant, according to whether it replaces or does not replace some other cause of mortality.

It is important to recognize that the percentage of parasitism means little, from a control standpoint, unless correlated with host population density. It is obvious that 50 per cent destruction when there are 100 mealybugs per tree gives a more satisfactory control than 90 per cent when there are 1,000 mealybugs per tree; yet esti-

mates of the importance of parasites are almost invariably given in percentage of hosts attacked with no reference to the host population density.

There is a decided tendency for the percentage of parasitism to increase as host density increases, and to decrease as host density decreases. For this reason a parasite which is capable of destroying 99 per cent of its host where the latter is abundant, may, by its own effectiveness, so reduce the host population that the percentage of parasitism drops to a relatively low figure. It is a mistake, therefore, to assume, because a parasite is destroying only a small percentage of its host when the host is scarce, that the parasite has no important effect on the maintenance of a low host population density. It may be *the important* factor.

When the parasites of the citrophilus mealybug were first introduced the host population density was high, and very few mealybugs in the heavily infested groves escaped attack. As the mealybug population declined, there has been a corresponding decline in the percentage of parasitism, as shown by the average sample.

ECONOMIC EFFECT OF INTRODUCED PARASITES

Since the discovery of *Pseudococcus gahani* Green in California in 1913, this mealybug has gradually and with considerable rapidity spread until it is now generally distributed in most of the regions in which it is capable of developing. In southern California it became a major pest of citrus trees and fruit, while in the northern part of the state it was the most troublesome pest of ornamentals and in some localities did considerable damage to deciduous fruits, particularly pears and apples.

In spite of the efficient work of the local insectaries in the mass production and distribution of *Cryptolaemus montrousieri,* the number of groves in which the control was unsatisfactory increased each year. As stated in the introduction, in 1927 the condition became so serious as to make it necessary to find a means of improving the situation. This led to the exploration in Australia and to the discovery and successful introduction into California of the insect enemies of the citrophilus mealybug discussed in this paper.

During the summer of 1928, after the establishment of the parasites, there was a rapid increase and dissemination of both *Coccophagus* and *Tetracnemus,* and in the spring of 1929 there was a very appreciable reduction in the number of mealybugs in the districts

where the parasites had been thoroughly established. During 1929 there was carried out, largely by certain local insectaries, an extensive production and distribution of *Coccophagus* and to some extent of *Tetracnemus,* so that by the spring of 1930 practically the entire infested citrus acreage in southern California had been colonized and the parasites thoroughly established. In the spring of 1930, at the time of the year when the so-called 'peak hatch' of mealybugs had usually taken place, this phenomenon failed to occur, the parasites having so reduced the overwintering mealybugs that the spring hatch was insignificant from an economic point of view. Throughout the following year, 1931, this favorable condition has been maintained; no infestations of any economic importance have occurred in any of the areas where the parasites have been thoroughly established, and this now includes all of the infested citrus districts of southern California. In northern California, around San Francisco Bay, where this mealybug had been a nuisance in gardens and parks, the establishment in 1929 of these parasites has resulted in control of the pest. It is now found only on occasional plants which are heavily infested with Argentine ants. In the Monterey Bay region, however, where the parasites have only recently been released and in very small numbers, the citrophilus mealybug infestation has been so heavy as to kill a considerable amount of wild growth in the hills, and has been so abundant on shade trees in at least one city that the fire department has been engaged in washing the honeydew from the sidewalks and streets.

There is no method known at the present time for measuring accurately the quantitative effect of separate environmental factors on the population density of a phytophagous insect. Conclusions must still be based on general field observation, and the contention that the disappearance of injurious infestations of the citrophilus mealybug is due to the work of *Coccophagus* and *Tetracnemus* is based on the observation that without exception the absence or occurrence of serious infestations of the pest has been positively correlated with the presence or absence of the parasites. During the past three years there has been a sufficient number of heavy infestations in localities where the parasites were not present to give reliability to the conclusions. These infestations served as check plots and demonstrated that the generally low population level of the mealybug was a result of the work of the parasites and not due to climatic influences unfavorable to the mealybugs. Invariably when the parasites later became well established in these localities, the mealybug population level took a decided, and apparently permanent, drop.

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